03C0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

OIPE JCIT DEC 19 2001 PATENT & TRADEMARK OFFICE

#4

ATTY.'S DOCKET: MOESTRUP=1A

In re Application of:) Art Unit:

Søren MOESTRUP et al) Examiner:

Appln. No.: 09/977,577) Washington, D.C.

Filed: October 16, 2001)

For: THE FUNCTION OF A HAPTOGLOBIN-HAEMOGLOBIN...) December 19, 2001

REQUEST FOR PRIORITY

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with the provisions of 37 CFR §1.55 and the requirements of 35 U.S.C. §119, filed herewith a certified copy of:

Denmark Appln. No.:PA 2001 00039	Filed: January 11, 2001
Denmark Appln. No.:PA 2000 01543	Filed: October 16, 2000

It is respectfully requested that applicant be granted the benefit of the priority date of the foreign application.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By [signature]
Iver P. Cooper
Registration No. 20,005

IPC:jmb
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528

f:/,h/hoib/moestrup1a/PriorityDocPTOCoverLtr19dec01.doc

Kongeriget Danmark

Patent application No.: PA 2000 01543

Date of filing: 16 October 2000

Applicant: ProteoPharma ApS
c/o Østjysk Innovation
Forskerparken, Gustav Wieds Vej 10
DK-8000 Århus C

This is to certify the correctness of the following information:

The attached photocopy is a true copy of the following document:

- The specification, claims and figures as filed with the application on the filing date indicated above.

Patent- og
Varemærkestyrelsen
Erhvervsministeriet

Taastrup, 11 December 2001

Karin Schlichting
Head Clerk

The function of a haptoglobin-haemoglobin receptor and the uses thereof

The present invention relates to haptoglobin-haemoglobin (Hp-Hb) complex or a part thereof or a mimic thereof being operably linked to a substance and capable of binding a CD163 receptor. Furthermore, the invention relates to a CD163 variant capable of binding at least one haptoglobin-haemoglobin (Hp-Hb) complex, and the use of the Hp-Hb complex and the CD163 receptor for therapy.

Background of the invention

Normal adult haemoglobin consists of a tetramer of four haemoglobin chains, two α-chains and two β-chains. O_2 binds to the tetrameric form of haemoglobin and is transported in the blood. Fetal blood comprises fetal haemoglobin, a tetramer consisting of two α-chains and two γ-chains. Further haemoglobin chains have been identified, such as δ-chains, ε-chains, zeta-chains, τ-chains or the S form known to be the mutation seen in haemoglobin of individuals suffering from sickle cell disease.

Intravascular lysis of red blood cells (haemolysis) leads to the release of haemoglobin into plasma. This phenomenon occurs during physiological as well as pathological conditions. Pathological complications are severe when accelerated in infectious e.g. malaria), inherited (e.g. sicle cell anemia), or autoimmune diseases. The haemoglobin tetramers are converted to haemoglobin dimers capable of binding haptoglobin. In the plasma haemoglobin is captured by the acute phase protein haptoglobin. Haptoglobin is a blood plasma protein having a molecular weight of approximately 86.000 to 400.000 and plays an important role in the metabolism of haemoglobin liberated into the blood stream. When liberated excessively in the blood the haemoglobin is excreted into the urine through the renal tubules, resulting in not only an iron loss but also disorders of the renal tubules. Because haptoglobin binds selectively and firmly to haemoglobin in vivo and thereby forms a haemoglobin-haptoglobin complex, it has important functions in the recovery of iron and in the prevention of renal disorders.

Hp is synthesised as a single chain, which is post-translationally cleaved into an amino-terminal α chain and a carboxy-terminal β chain. The basic structure of Hp,

as found in most mammals, is a homodimer (Fig. 2a), in which th two Hp molecules are linked by a single disulfide bond via their respective ~9 kDa α chains. In man, a variant with a long α chain is also present in all populations. This variant arose apparently by an early intragenic duplication, presumably originating from an unequal crossover of two basic alleles, resulting in an Hp with an α chain of ~14 kDa. The short and long α chains are designated as α^1 and α^2 respectively. Since the cysteine forming the intermolecular disulfide bond between the α chains is also duplicated, humans carrying the long variant allele exhibit a multimeric Hp phenotype (Fig. 2a).

Conventional human haptoglobins have been well studied; they were discovered over 40 years ago and their role is thought to be in the plasma transport of free haemoglobin. Additionally, haptoglobin is believed to have anti-inflammatory activities, such as its decreasing effect on neutrophil metabolism, and an effect on the immune system by possibly modulating B cell proliferation and decrease antibody production. The mechanisms of the influence of haptoglobin on immune function is unknown. The potential signalling pathways by which haptoglobin is mediating its effects, and the existence of a haptoglobin receptor have not been disclosed in the prior art.

However, Ghmati et al., 1996 describe a study in which haptoglobin is an alternative low-affinity ligand for CD11b/CD18 on monocyte cell lines. CD11b/CD18 is part of the integrin family and is involved in inflammatory and immunological functions.

Yet another receptor molecule present on monocytes is CD163. It is identified as a member of the scavenger receptor cystein-rich superfamily (SRCR) present on cells of the monocytic family, such as most macrophages. Ritter et al., 1999 discuss the regulation, promoter structure and genomic organisation of the CD163 receptor. The precise function of CD163 is not disclosed. Furthermore, previous work on the biological function of CD163 is limited to a study on the effect of antibody-mediated crosslinking of CD163 on cultured monocytes(Van den Heuvel, M.M. et al. Regulation of CD163 on human macrophages: cross-linking of CD163 induces signalling and activation. J. Leukoc.Bil. 66, 858-866 (1999). The CD163 surface ligation induces a tyrosine kinase dependent signal resulting in intracellular calcium mobilisa-

tion, inositol triphosphate production, and increased secretion of anti-inflammatory cytokines.

Summary

The present inventors have identified CD163 as the high-affinity macrophage receptor for haptoglobin-haemoglobin complexes and the present invention relates to the use of the CD163 receptor and/or a CD163 variant, and/or the use of haptoglobin-haemoglobin complexes in the diagnosis, prevention and/or treatment of various diseases and disorders.

Accordingly, the invention describes a Hp-Hb complex, or a part thereof or a mimic thereof being operably linked to a substance, wherein the Hp-Hb complex is capable of binding CD163 and/or a CD163 variant. In the present context the term Hp-Hb complex includes a functional equivalent thereof unless expressively otherwise stated.

In the present context the term "substance" means a component heterologous to the Hp-Hb complex, such as a drug, a gene, a vesicle, a vector, or the like.

Further, the invention concerns the use of at least one Hp-Hb complex for the delivery of at least one drug, or at least one gene to a cell expressing a CD163 receptor and/or a CD163 receptor variant. The invention also relates to the use of at least one Hp-Hb complex, further comprising a CD163 receptor variant for the identification of at least one Hp-Hb complex in serum and/or plasma of an individual.

In the present context the term CD163 receptor is used in its conventional meaning as the scavenger receptor CD163 of monocytes and most tissue macrophages. The term CD163 is used synonymously with the term CD163 receptor.

The term a CD163 receptor variant is used synonymously with the term CD163 variant.

In another aspect, the present invention relates to a CD163 variant capable of binding at least one haptoglobin-haemoglobin (Hp-Hb) complex.

In a further aspect of the invention the use of at least one CD163 variant in the manufacture of a medicament for treatment of disorders/complications related to haemolysis in an individual in need of such treatment is disclosed.

Also, the invention describes the use of at least one CD163 variant for the removal of at least one Hp-Hb complex in serum and/or plasma of an individual, and the use for the determination of the haemolysis rate of an individual. Further, the use of at least one complex comprising haemoglobin and haptoglobin as a marker for a cell expressing a CD163 variant, wherein at least one of the haemoglobin or haptoglobin molecules are labelled is also described in the present invention.

An object of the invention is to provide a CD163 molecule for the use as a medicament. The areas of use of a CD163 molecule according to the invention are identical to the areas of use described above for the CD163 variant.

Further, a Hp-Hb complex, or a part thereof or a mimic thereof being operably linked to a substance, wherein the Hp-Hb complex is capable of binding said CD163 molecule is also within the scope of the invention.

In the present context the word medicament is used in its normal meaning as a composition to be administered to an individual for prophylactic, therapeutic and/or diagnostic purposes.

Figures

Fig. 1: is an illustration of the steps involved in the Hp-Hb/CD163 binding.

Fig. 2: shows examples of a) a haptoglobin dimer, b) haptoglobin multimers, c) Hp-Hb complexes, and d) a SDS-PAGE gel of mono- and multimers of haptoglobin.

Fig. 3: shows a CD163 molecule.

Fig. 4: shows 9 different haptoglobin sequences

Fig. 5: shows 4 different CD163 sequences

Fig. 6: Binding of Hp-Hb to CD163. a, Illustration of the subunit organisation and disulfide bridging of the various Hp and Hp-Hb complexes. The inset shows non-reducing SDS-PAGE of the Hp(1-1) dimer and Hp(2-2) multimers. b, Surface plasmon resonance analysis of the binding of Hp-Hb to CD163. The measurements were carried out at Hb concentrations ranging from zero to 100 µg/ml in the absence of Hp (left panel), or in the presence of 50 µg/ml of Hp(1-1) (middle panel), and 50 µg/ml Hp(2-2) (right panel). No binding was observed with either Hb or Hp alone, and saturation of the binding was obtained at 50 µg/ml Hb for both Hp phenotypes. c, Inhibition of CD163-binding of ^{125}I-labelled Hp(1-1)-Hb (left panels) and Hp(2-2)-Hb (right panels) by polyclonal anti-CD163 IgG, non-immune rabbit IgG, EDTA (5 mM) and by various concentrations of unlabelled Hp(1-1)-Hb and Hp(2-2)-Hb complexes. CD163 was immobilised in microtiter plate wells.

Fig. 7: CD163-mediated endocytosis of ^{125}I-Hp-Hb. a, Cell-association and degradation of ^{125}I-Hp(2-2)-Hb in mock-transfected (left panel) and CD163 cDNA-transfected CHO cells (middle panel). Addition of the lysosomal inhibitors chloroquine and leupeptin (both 100 µM) inhibited degradation leading to cellular accumulation of radioactivity (right panel). b, Inhibition of ^{125}I-Hp-Hb uptake in CD163 cDNA-transfected CHO cells (left panel) and in CD163-expressing histiocytic lymphoma-derived SU-DHL-1 cells (right panel). Both cell types displayed a saturable uptake inhibited by anti-CD163 polyclonal IgG. The insets in a and b show anti-CD163 immunoblotting of the cells.

Detailed description of the invention

In a first aspect the present invention relates to a Hp-Hb complex or a functional equivalent thereof being operably linked to a substance, said complex and/or functional equivalent thereof being capable of binding to a CD163 receptor and/or a CD163 variant. A functional equivalent of a Hp-Hb complex is to be understood as any part (or fragment) or any mimic capable of binding to a CD163 receptor.

"Functional equivalency" as used in the present invention is according to one preferred embodiment established by means of reference to the corresponding functionality of a predetermined Hp-Hb fragment.

In the present context the term "Hp-Hb complex" means a complex of at least one haptoglobin chain and at least one haemoglobin chain. Preferably the complex comprises at least one haptoglobin chain and at least one dimeric form of haemoglobin chains. In a further preferred embodiment the complex comprises a multimeric form of haptoglobin chains such as a dimeric form, each haptoglobin chain binding at least one haemoglobin chain, preferably a dimer of haemoglobin chains.

The fragment thereof should be understood to be any part of the Hp-Hb complex capable of binding to the CD163 receptor or to a variant thereof and through said binding activate uptake of the fragment and/or the substance into the CD163 presenting cell.

The mimic thereof should be understood to be any modification of the Hp-Hb complex (in the present context also called a variant of the complex) or any other molecule capable of binding to the CD163 receptor or to a variant thereof and through said binding activating uptake of the fragment and/or the substance into the CD163 presenting cell. Mimics may be peptides, peptide derivatives, antibodies, as well as non-peptide compounds, such as small organic compounds, sugars and fats.

Fragments and/or mimics may be identified by combinatorial chemistry using the CD163 receptor.

The Hp-Hb complex fragment or mimic is preferably, capable of binding to a region in the SRCR domains I-IX of the CD163 receptor, such as capable of binding to a region in the SRCR domains I-VIII of the CD163 receptor, capable of binding to a region in the SRCR domains I-VII of the CD163 receptor, capable of binding to a region in the SRCR domains I-VI of the CD163 receptor, capable of binding to a region in the SRCR domains I-V of the CD163 receptor, capable of binding to a region in the SRCR domains I-IV of the CD163 receptor, capable of binding to a re-

gion in the SRCR domains I-III of the CD163 receptor, capable of binding to a region in the SRCR domains I-II of the CD163 receptor, or a variant thereof.

It is preferred that the Hp-Hb complex or a part thereof or a mimic thereof is available in a purified and/or isolated form.

According to the invention the term "Hp-Hb complex" is meant to include functional equivalents of the Hp-Hb complex comprising a predetermined amino acid sequence. In the present context the term "predetermined amino acid sequence of Hp-Hb complex" relates to both the haptoglobin sequence and the haemoglobin sequence.

The predetermined sequence of a haptoglobin chain may be any of the sequences shown in Fig. 4a and 4b, i.e. any of the sequences having the sequence identification in the sequence database SWISS-PROT (sp) or trEMBL (tr).

sp|P00737|HPT1_HUMAN
sp|P00738|HPT2_HUMAN
sp|P50417|HPT_ATEGE
tr|Q60574|Q60574
tr|Q61646|Q61646
sp|Q62558|HPT_MUSSA
sp|P06866|HPT_RAT
tr|O35086|O35086
sp|P19006|HPT_CANFA

A predetermined amino acid sequence for a heamoglobin chain may be any of the sequences mentioned below together with accession No. in the sequence database SWISSPROT:

sp|P01922|HBA_HUMAN HEMOGLOBIN ALPHA CHAIN –
Homo sapiens (Human), Pan troglodytes (Chimpanzee), and Pan paniscus (Pygmy chimpanzee) (Bonobo).

VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSH

GSAQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKL
LSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR

sp|P02023|HBB_HUMAN HEMOGLOBIN BETA CHAIN –
Homo sapiens (Human), Pan troglodytes (Chimpanzee), and Pan paniscus (Pygmy chimpanzee) (Bonobo).

VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLST
PDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDP
ENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH

sp|P02042|HBD_HUMAN HEMOGLOBIN DELTA CHAIN –
Homo sapiens (Human).

VHLTPEEKTAVNALWGKVNVDAVGGEALGRLLVVYPWTQRFFESFGDLSS
PDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFSQLSELHCDKLHVDP
ENFRLLGNVLVCVLARNFGKEFTPQMQAAYQKVVAGVANALAHKYH

sp|P02096|HBG_HUMAN HEMOGLOBIN GAMMA-A AND GAMMA-G CHAINS -
Homo sapiens (Human), and Pan troglodytes (Chimpanzee).

GHFTEEDKATITSLWGKVNVEDAGGETLGRLLVVYPWTQRFFDSFGNLSS
ASAIMGNPKVKAHGKKVLTSLGDAIKHLDDLKGTFAQLSELHCDKLHVDP
ENFKLLGNVLVTVLAIHFGKEFTPEVQASWQKMVTAVASALSSRYH

sp|P09105|HBAT_HUMAN HEMOGLOBIN THETA-1 CHAIN –
Homo sapiens (Human).

ALSAEDRALVRALWKKLGSNVGVYTTEALERTFLAFPATKTYFSHLDLSP
GSSQVRAHGQKVADALSLAVERLDDLPHALSALSHLHACQLRVDPASFQL
LGHCLLVTLARHYPGDFSPALQASLDKFLSHVISALVSEYR

sp|P02008|HBAZ_HUMAN HEMOGLOBIN ZETA CHAIN –
Homo sapiens (Human).

SLTKTERTIIVSMWAKISTQADTIGTETLERLFLSHPQTKTYFPHFDLHP
GSAQLRAHGSKVVAAVGDAVKSIDDIGGALSKLSELHAYILRVDPVNFKL
LSHCLLVTLAARFPADFTAEAHAAWDKFLSVVSSVLTEKYR

sp|P02100|HBE_HUMAN HEMOGLOBIN EPSILON CHAIN –
Homo sapiens (Human).

VHFTAEEKAAVTSLWSKMNVEEAGGEALGRLLVVYPWTQRFFDSFGNLSS
PSAILGNPKVKAHGKKVLTSFGDAIKNMDNLKPAFAKLSELHCDKLHVDP
ENFKLLGNVMVIILATHFGKEFTPEVQAAWQKLVSAVAIALAHKYH

tr|Q14510|Q14510 SICKLE BETA-HEMOGLOBIN MRNA –
Homo sapiens (Human).

MVHLTPVEKSAVTAXWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLS
TPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVD
PENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH

A "functional equivalent" is defined as:

i) equivalents comprising an amino acid sequence capable of being recognised by an antibody also capable of recognising the predetermined amino acid sequence, and/or

ii) equivalents comprising an amino acid sequence capable of binding to a receptor moiety also capable of binding the predetermined amino acid sequence, and/or

iii) equivalents having at least a substantially similar or higher binding affinity to CD163 as at least a monomeric Hp-Hb complex comprising said predetermined amino acid sequence.

According to the present invention a functional equivalent of a Hp-Hb complex or fragments thereof may be obtained by addition, substitution or deletion of at least one amino acid in either or both of the haptoglobin sequence and the haemoglobin sequence. Thus, a functional equivalent of the Hp-Hb complex may comprise a modification of either of the components of the complex or both.

When the amino acid sequence comprises a substitution of one amino acid for another, such a substitution may be a conservative amino acid substitution. Fragments of the complex according to the present invention may comprise more than one such substitution, such as e.g. two conservative amino acid substitutions, for example three or four conservative amino acid substitutions, such as five or six conservative amino acid substitutions, for example seven or eight conservative amino acid substitutions, such as from 10 to 15 conservative amino acid substitutions, for example from 15 to 25 conservative amino acid substitution. Substitutions can be made within any one or more groups of predetermined amino acids.

Examples of equivalents comprising one or more conservative amino acid substitutions including one or more conservative amino acid substitutions within the same group of predetermined amino acids, or a plurality of conservative amino acid substitutions, wherein each conservative substitution is generated by substitution within a different group of predetermined amino acids.

Accordingly, mimics of the complex, or fragments thereof according to the invention may comprise, within the same mimic, or fragments thereof or among different mimics, or fragments thereof, at least one substitution, such as a plurality of substitutions introduced independently of one another. Mimics of the complex, or fragments thereof may thus comprise conservative substitutions independently of one another, wherein at least one glycine (Gly) of said mimic, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and Ile, and independently thereof, mimics, or fragments thereof, wherein at least one of said alanines (Ala) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Val, Leu, and Ile, and independently thereof, mimics, or fragments thereof, wherein at least one valine (Val) of said mimic, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala,

Leu, and Ile, and independently thereof, mimics, or fragments thereof, wherein at least one of said leucines (Leu) of said mimic, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and Ile, and independently thereof, mimics, or fragments thereof, wherein at least one isoleucine (Ile) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, mimics, or fragments thereof wherein at least one of said aspartic acids (Asp) of said mimic, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Glu, Asn, and Gln, and independently thereof, mimics, or fragments thereof, wherein at least one of said phenylalanines (Phe) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, mimics, or fragments thereof, wherein at least one of said tyrosines (Tyr) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, mimics, or fragments thereof, wherein at least one of said arginines (Arg) of said fragment is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, mimics, or fragments thereof, wherein at least one lysine (Lys) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, mimics, or fragments thereof, wherein at least one of said aspargines (Asn) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, mimics, or fragments thereof, wherein at least one glutamine (Gln) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and independently thereof, mimics, or fragments thereof, wherein at least one proline (Pro) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, mimics, or fragments thereof, wherein at least one of said cysteines (Cys) of said mimics, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

It is clear from the above outline that the same equivalent or fragment thereof may comprise more than one conservative amino acid substitution from more than one group of conservative amino acids as defined herein above.

Conservative substitutions may be introduced in any position of a preferred predetermined Hp-Hb complex of fragment thereof. It may however also be desirable to introduce non-conservative substitutions, particularly, but not limited to, a non-conservative substitution in any one or more positions.

A non-conservative substitution leading to the formation of a functionally equivalent fragment of the sequences in Figure 1 or 2 would for example i) differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

Substitution of amino acids may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like. Exemplary amino acid substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

The addition or deletion of an amino acid may be an addition or deletion of from 2 to preferably 10 amino acids, such as from 2 to 8 amino acids, for example from 2 to 6 amino acids, such as from 2 to 4 amino acids. However, additions of more than 10

amino acids, such as additions from 10 to 200 amino acids, are also comprised within the present invention. In the discussion of deletions and additions reference is made to a monomeric form of the complex, i.e. two haemoglobin chains and one haptoglobin chain. In the multimeric forms additions/deletions may be made individually in each monomer of the multimer.

It will thus be understood that the invention concerns Hp-Hb complexes comprising at least one fragment capable of binding at least one CD163 receptor or a variant thereof, including any variants and functional equivalents of such at least one fragment.

The Hp-Hb complex according to the present invention, including any functional equivalents and fragments thereof, may in one embodiment comprise less than 300 amino acid residues, such as less than 275 amino acid residues, such as less than 250 amino acid residues, such as less than 225 amino acid residues, such as less than 200 amino acid residues, such as less than 175 amino acid residues, such as less than 150 amino acid residues, such as less than 125 amino acid residues, such as less than 100 amino acid residues, such as less than 95 amino acid residues, for example less than 90 amino acid residues, such as less than 85 amino acid residues, for example less than 80 amino acid residues, such as less than 75 amino acid residues, for example less than 70 amino acid residues, such as less than 65 amino acid residues, for example less than 60 amino acid residues, such as less than 55 amino acid residues, for example less than 50 amino acid residues, such as less than 45 amino acid residues, for example less than 40 amino acid residues, such as less than 38 amino acid residues, for example less than 37 amino acid residues, such as less than 36 amino acid residues, for example less than 35 amino acid residues, such as less than 34 amino acid residues, for example less than 33 amino acid residues, such as less than 32 amino acid residues, for example less than 31 amino acid residues, such as about 30 amino acid residues, for example less than 30 amino acid residues, such as about 29 amino acid residues. The number of amino acid residues relate to the total number of amino acid residues in the complex independent of the complex being a linear amino acid sequence or a non-linear complex of amino acid sequences.

A fragment comprising the CD163 binding region of native Hp-Hb complex is particularly preferred. However, the invention is not limited to fragments comprising the CD163 receptor binding region. Deletions of such fragments generating functionally equivalent fragments of the complex comprising less than the CD163 receptor binding region are also comprised in the present invention. Functionally equivalent complex peptides, and fragments thereof according to the present invention, may comprise less or more amino acid residues than CD163 receptor binding region.

Fragments comprising the CD163 receptor binding region of HP-Hb complex preferably comprises regions capable of binding to the SRCR domains I-IX of the CD163 receptor, such as capable of binding to a region in the SRCR domains I-VIII of the CD163 receptor, capable of binding to a region in the SRCR domains I-VII of the CD163 receptor, capable of binding to a region in the SRCR domains I-VI of the CD163 receptor, capable of binding to a region in the SRCR domains I-V of the CD163 receptor, capable of binding to a region in the SRCR domains I-IV of the CD163 receptor, capable of binding to a region in the SRCR domains I-III of the CD163 receptor, capable of binding to a region in the SRCR domains I-II of the CD163 receptor.

Fragments of the complex prefereably comprises at least the haevy chain (β) of haptoglobin or a part of said chain capable of forming complex with haemoglobin.

In particular the fragments may comprise a sequence corresponding to aa 103-347 of sp|P00737 in Fig. 4 or to aa 162-406 of sp|P00738.

In one embodiment mimics may be understood to exhibit amino acid sequences gradually differing from the preferred predetermined sequence, as the number and scope of insertions, deletions and substitutions including conservative substitutions increases. This difference is measured as a reduction in homology between the predetermined sequence and the mimic.

All functional equivalents of Hp-Hb complexes are included within the scope of this invention, regardless of the degree of homology that they show to a predetermined sequence of Hp-Hb complexes. The reason for this is that some regions of the com-

plex are most likely readily mutatable, or capable of being completely deleted, without any significant effect on the binding activity of the resulting fragment.

A functional equivalent obtained by substitution may well exhibit some form or degree of native Hp-Hb activity, and yet be less homologous, if residues containing functionally similar amino acid side chains are substituted. Functionally similar in this respect refers to dominant characteristics of the side chains such as hydrophobic, basic, neutral or acidic, or the presence or absence of steric bulk. Accordingly, in one embodiment of the invention, the degree of identity between i) a given complex equivalent capable of effect and ii) a preferred predetermined fragment, is not a principal measure of the fragment as a variant or functional equivalent of a preferred predetermined complex fragment according to the present invention.

Fragments sharing at least some homology with a preferred predetermined complex fragment of at least 50 amino acids, more preferably at least 100 amino acids, are to be considered as falling within the scope of the present invention when they are at least about 40 percent homologous with the preferred predetermined Hp-Hb complex or fragment thereof, such as at least about 50 percent homologous, for example at least about 60 percent homologous, such as at least about 70 percent homologous, for example at least about 75 percent homologous, such as at least about 80 percent homologous, for example at least about 85 percent homologous, such as at least about 90 percent homologous, for example at least 92 percent homologous, such as at least 94 percent homologous, for example at least 95 percent homologous, such as at least 96 percent homologous, for example at least 97 percent homologous, such as at least 98 percent homologous, for example at least 99 percent homologous homologous with the predetermined complex fragment. In a preferred embodiment the above percentages for homology also relates to percentage identity.

The Hp-Hb complex is preferably constituted of at least two different chains (sequences) wherein one chain constitutes the haptoglobin part of the complex and the other chain constitutes the haemoglobin part. A mimic of the Hp-Hb complex may however be constituted by one chain (sequence) or multimers of said chain, wherein the chain is a steric equivalent of the Hp-Hb complex.

In addition to the mimics described herein, sterically similar variants may be formulated to mimic the key portions of the variant structure and that such compounds may also be used in the same manner as the variants of the invention. This may be achieved by techniques of modelling and chemical designing known to those of skill in the art. It will be understood that all such sterically similar constructs fall within the scope of the present invention.

In one embodiment the Hp-Hb complex or parts thereof or mimics thereof is synthesised by automated synthesis. Any of the commercially available solid-phase techniques may be employed, such as the Merrifield solid phase synthesis method, in which amino acids are sequentially added to a growing amino acid chain. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied Biosystems, Inc. of Foster City, Calif., and may generally be operated according to the manufacturer's instructions. Solid phase synthesis will enable the incorporation of desirable amino acid substitutions into any Hp-Hb complex according to the present invention. It will be understood that substitutions, deletions, insertions or any subcombination thereof may be combined to arrive at a final sequence of a functional equivalent. Insertions shall be understood to include amino-terminal and/or carboxyl-terminal fusions, e.g. with a hydrophobic or immunogenic protein or a carrier such as any polypeptide or scaffold structure capable as serving as a carrier.

Hp-Hb complexes according to the invention may be synthesised both in vitro and in vivo. Methods for in vitro synthesis are well known. When synthesized in vivo, a host cell is transformed with vectors containing DNA encoding various parts of the Hp-Hb complex. A vector is defined as a replicable nucleic acid construct. Vectors are used to mediate expression of the Hp-Hb complex. An expression vector is a replicable DNA construct in which a nucleic acid sequence encoding the predetermined Hp-Hb complex, or any functional equivalent thereof that can be expressed in vivo, is operably linked to suitable control sequences capable of effecting the expression of the variant, or equivalent in a suitable host. Such control sequences are well known in the art.

A DNA sequence encoding the various parts of the Hp-Hb complex is meaning a DNA sequence encoding the haptoglobin part and a DNA sequence encoding the

haemoglobin part. In another embodiment the DNA sequence may be one s quence encoding one peptide sequence which post-translationally is cleaved into the haptoglobin part and the haemoglobin part. In yet another embodiment one peptide constituting both parts is not cleaved, but due to post-translationally folding and/or processing functions as the complex.

Accordingly, one aspect of the invention relates to a DNA sequence encoding a Hp-Hb complex as defined above, the DNA sequence may be a genomic DNA sequence, a cDNA sequence or a mixture of a genomic and a cDNA sequence.

Furthermore, the invention relates to a vector comprising the DNA sequence, as well as to a cell comprising said vector, said cell being capable of expressing the DNA sequence, either as a Hp-Hb complex released into the cell culturing media, or a Hp-Hb complex anchored to the cell membrane.

Cultures of cells may be derived from prokaryotic and eukaryotic cells. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture but human cells are preferred. Examples of useful host cell lines E.coli, yeast, or human cell lines. Preferred host cells are eukaryotic cells known to synthesize endogenous haptoglobin and/or haemoglobin. Cultures of such host cells may be isolated and used as a source of the variant, or used in therapeutic methods of treatment, including therapeutic methods aimed at diagnostic methods carried out on the human or animal body.

In order to increase the binding affinity the Hp-Hb complex or part thereof or mimic thereof is preferably dimeric. In a more preferred embodiment the the Hp-Hb complex or a part thereof or a mimic thereof is multimeric. Dimeric and multimeric relates to the number of haptoglobin monomers. The haemoglobin may be monomeric or dimeric for each haptoglobin chain.There is a correlation between the type of multimeric forms of the Hp-Hb complex and the degree of binding to a CD163 receptor or a CD163 variant of the invention. A multimeric form of a Hp-Hb complex will due to its size have an increased exposure of encountering CD163 variants as when compared to a monomeric, or even a dimeric form, and thus an increased functional affinity to CD163 variants is observed. Furthermore, the multimeric form of the com-

plex may bind to more than one receptor on the CD163 presenting cell leading to increased avidity of the binding.

The multimers may be created by a common linker moiety, such as S-S bridges as in the naturally occurring haptoglobin. The common linker moiety, is preferably located so that complex-forming with haemoglobin is not disturbed. It is preferred that the common linker moiety is located in the light chain of haptoglobin.

According to the invention the Hp-Hb complex, or a part thereof being operably linked to a substance as described above may be for the use as a medicament. Such medicament may operate through a method, wherein the Hp-Hb complex or a part thereof is used in a method of treatment of an individual, comprising the steps of:

i) providing a Hp-Hb complex, or a part thereof or a mimic thereof capable of binding to the CD163 receptor and/or the CD163 variant,

ii) operably linking a substance as defined above to the Hp-Hb complex or a part thereof or mimic thereof,

iii) administering the medicament comprising the substance operably linked to the Hp-Hb complex to an individual in need thereof.

The term operably linked means that the substance is coupled or bound to the complex in a manner so that the substance is transported to the cell presenting a CD163 receptor or a CD163 variant, whereafter the substance may be released from the complex if appropriate.

Due to the binding of the complex or fragment or mimic thereof to the CD163 receptor and/or a CD163 variant the substance comprised in or bound to the Hp-Hb complex is either taken up by the CD163 presenting cells or at least located in the environment close to the cells. Thereby it is possible to concentrate the substance in or around the cell presenting the CD163 receptor. A test for analysing optional uptake is described below in Example 4.

In one embodiment of the invention th Hp-Hb complex, or a part thereof may be operably linked to a substance, such as a medicament, a gene, a vesicle, vector or the like.

The medicament may be any medicament for which it is desirable to target the drug to a particular tissue or particular cells. In particular the medicament is an antimicrobial agent or a cancer drug.

The medicament is preferably a medicament against diseases in relation to monocytes, such as macrophages. In particular the invention relates to a complex being operably linked to a anti-HIV drug.

In another embodiment the substance is a medicament against lymphomas, such as histiocytic lymphomas.

In yet another embodiment the substance may stimulate the macrophages to produce interleukin 6.

In a further embodiment the substance is an antigen for vaccine purposes.

In another embodiment the substance of the Hp-Hb complex, or a functional equivalent thereof comprises a gene, i.e. a gene construct. The gene may be any gene encoding a particular biological function. For example the gene may comprise a nucleic acid, such as PNA, LNA, DNA or RNA, or the gene may comprise cDNA. The Hp-Hb complex comprising a gene may be used in gene-delivery therapy, whereby the gene is taken up by the cell presenting the CD163 receptor or a variant thereof.

The constructs can be introduced as one or more DNA molecules or constructs. The constructs are prepared in conventional ways, where the genes and regulatory regions may be isolated, as appropriate, ligated, cloned in an appropriate cloning host, analyzed by restriction or sequencing, or other convenient means. Using PCR, individual fragments including all or portions of a functional unit may be isolated, where one or more mutations may be introduced using "primer repair", ligation, in vitro mutagenesis, etc. as appropriate. The construct(s) once completed and demon-

strated to have the appropriate sequences may then be introduced int host cells by any convenient means, as discussed in more detail below.

The constructs may be introduced as a single DNA molecule encoding all of the genes, or different DNA molecules having one or more genes. The constructs may be introduced simultaneously or consecutively, each with the same or different markers.

The gene may be linked to the complex as such or protected by any suitable system normally used for transfection such as viral vectors or artificial viral envelope, liposomes or micellas, wherein the system is linked to the complex.

Numerous techniques for introducing DNA into eukaryotic cells are known to the skilled artisan. Often this is done by means of vectors, and often in the form of nucleic acid encapsidated by a (frequently virus-like) proteinaceous coat. Gene delivery systems may be applied to a wide range of clinical as well as experimental applications.

Vectors containing useful elements such as selectable and/or amplifiable markers, promoter/enhancer elements for expression in mammalian, particularly human, cells, and which may be used to prepare stocks of construct DNAs and for carrying out transfections are well known in the art. Many are commercially available.

Various techniques have been developed for modification of target tissue and cells in vivo. A number of virus vectors, discussed below, are known which allow transfection and random integration of the virus into the host. See, for example, Dubensky et al. (1984) Proc. Natl. Acad. Sci. USA 81:7529-7533; Kaneda et al., (1989) Science 243:375-378; Hiebert et al. (1989) Proc. Natl. Acad. Sci. USA 86:3594-3598; Hatzoglu et al., (1990) J. Biol. Chem. 265:17285-17293; Ferry et al. (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381. Routes and modes of administering the vector include injection, e.g intravascularly or intramuscularly, inhalation, or other parenteral administration.

Advantages of adenovirus vectors for human gene therapy include the fact that recombination is rare, no human malignancies are known to be associated with such

viruses, the adenovirus genome is double stranded DNA which can be manipulated to accept foreign genes of up to 7.5 kb in size, and live adenovirus is a safe human vaccine organisms.

Another vector which can express the DNA molecule of the present invention, and is useful in gene therapy, particularly in humans, is vaccinia virus, which can be rendered non-replicating (U.S. Pat. Nos. 5,225,336; 5,204,243; 5,155,020; 4,769,330).

Based on the concept of viral mimicry, artificial viral envelopes (AVE) are designed based on the structure and composition of a viral membrane, such as HIV-1 or RSV and used to deliver genes into cells in vitro and in vivo. See, for example, U.S. Pat. No. 5,252,348, Schreier H. et al., J. Mol. Recognit., 1995, 8:59-62; Schreier H et al., J. Biol. Chem., 1994, 269:9090-9098; Schreier, H., Pharm. Acta Helv. 1994, 68:145-159; Chander, R et al. Life Sci., 1992, 50:481-489, which references are hereby incorporated by reference in their entirety. The envelope is preferably produced in a two-step dialysis procedure where the "naked" envelope is formed initially, followed by unidirectional insertion of the viral surface glycoprotein of interest. This process and the physical characteristics of the resulting AVE are described in detail by Chander et al., (supra). Examples of AVE systems are (a) an AVE containing the HIV-1 surface glycoprotein gp160 (Chander et al., supra; Schreier et al., 1995, supra) or glycosyl phosphatidylinositol (GPI)-linked gp120 (Schreier et al., 1994, supra), respectively, and (b) an AVE containing the respiratory syncytial virus (RSV) attachment (G) and fusion (F) glycoproteins (Stecenko, A. A. et al., Pharm. Pharmacol. Lett. 1:127-129 (1992)). Thus, vesicles are constructed which mimic the natural membranes of enveloped viruses in their ability to bind to and deliver materials to cells bearing corresponding surface receptors.

AVEs are used to deliver genes both by intravenous injection and by instillation in the lungs. For example, AVEs are manufactured to mimic RSV, exhibiting the RSV F surface glycoprotein which provides selective entry into epithelial cells. F-AVE are loaded with a plasmid coding for the gene of interest, (or a reporter gene such as CAT not present in mammalian tissue).

The AVE system described herein in physically and chemically essentially identical to the natural virus yet is entirely "artificial", as it is constructed from phospholipids,

cholesterol, and recombinant viral surface glycoproteins. Hence, th re is no carry-over of viral genetic information and no danger of inadvertant viral infection. Construction of the AVEs in two independent steps allows for bulk production of the plain lipid envelopes which, in a separate second step, can then be marked with the desired viral glycoprotein, also allowing for the preparation of protein cocktail formulations if desired.

Another delivery vehicle for use in the present invention are based on the recent description of attenuated Shigella as a DNA delivery system (Sizemore, D. R. et al., Science 270:299-302 (1995), which reference is incorporated by reference in its entirety). This approach exploits the ability of Shigellae to enter epithelial cells and escape the phagocytic vacuole as a method for delivering the gene construct into the cytoplasm of the target cell. Invasion with as few as one to five bacteria can result in expression of the foreign plasmid DNA delivered by these bacteria.

A preferred type of mediator of nonviral transfection in vitro and in vivo is cationic (ammonium derivatized) lipids. These positively charged lipids form complexes with negatively charged DNA, resulting in DNA charged neutralization and compaction. The complexes endocytosed upon association with the cell membrane, and the DNA somehow escapes the endosome, gaining access to the cytoplasm. Cationic lipid:DNA complexes appear highly stable under normal conditions. Studies of the cationic lipid DOTAP suggest the complex dissociates when the inner layer of the cell membrane is destabilized and anionic lipids from the inner layer displace DNA from the cationic lipid. Several cationic lipids are available commercially. Two of these, DMRI and DC-cholesterol, have been used in human clinical trials. First generation cationic lipids are less efficient than viral vectors. For delivery to lung, any inflammatory responses accompanying the liposome administration are reduced by changing the delivery mode to aerosol administration which distributes the dose more evenly.

The gene may be any gene appropriately expressed by the CD163 presenting cells. In one embodiment the gene may be a gene for CD163 as a gene therapy for individuals having reduced CD-163 expression.

In another embodiment the gene encodes an antigen for as a gene vaccination. In any situation it may be an advantage that macrophages do not multiply whereby this kind of gene therapy is an appropriate form of temporary gene therapy.

The gene therapy approach can be utilized in a site specific manner to deliver a retroviral vector to the tissue or organ of choice. Thus, for example, a catheter delivery system can be used (Nabel, E. G. et al., Science 244:1342 (1989)). Such methods, using either a retroviral vector or a liposome vector, is particularly useful to deliver the gene to a blood vessel wall.

Other virus vectors may also be used, in particular for human gene therapy, including recombinant adenovirus vectors.

A nontoxic and efficient method has recently been reported based on the Sendai virus, also known as hemagglutinating virus of Japan (HVJ). HVJ-liposome-mediated gene transfer is performed Morishita R et al., Hypertension (1993) 21:894-89.

Further, the substance of the Hp-Hb complex, or a part thereof may also comprise a tracer or a marker, such as chromophores, fluorphores, biotin, isotopes, enzymes, for identifying the cells presenting the CD163 receptor or a variant thereof. Thereby Hp-Hb complex may be used for diagnostic purposes as well.

In one embodiment the Hp-Hb complex or fragment thereof or mimic thereof being operably linked to a substance is capable of binding a CD163 variant only, in order to avoid binding to the naturally occuring CD163 receptor on macrophages. Thereby it is possible to direct a substance to a subgroup of cells presenting the CD163 variant only.

It is another object of the present invention to use a CD163 molecule as a medicament. Use of a CD163 molecule in the manufacture of a medicament for treatment of haemolysis in an individual in need of such treatment. There are a number of application fields, wherein one is the use of a CD163 molecule for the removal of at least one Hp-Hb complex in serum and/or plasma of an individual. A second application is the use of a CD163 molecule for the determination of the haemolysis rate

of an individual. Further, the use of at least ne complex comprising haemoglobin and haptoglobin as a marker for a cell, such as a macrophage expressing a CD163 molecule, wherein at least one of the haemoglobin or haptoglobin molecules are labelled is yet another application area.

According to the invention the term "CD163 variant" is meant to include functional equivalents of CD163, or a fragment of CD163, said CD163 comprising a predetermined amino acid sequence. A "variant" is defined as:

iv) variants comprising an amino acid sequence capable of being recognised by an antibody also capable of recognising the predetermined amino acid sequence, and/or

v) variants comprising an amino acid sequence capable of binding to a Hp-Hb complex also capable of binding the predetermined amino acid sequence, and/or

vi) variants having at least a substantially similar binding affinity to at least one Hp-Hb complex as said predetermined amino acid sequence.

By the term "predetermined amino acid sequence" is meant any of the amino acid sequences depicted in Figure 5a and 5b, i.e. any of the sequences for CD163 having the following sequence identification in sequence database trEMBL:

tr|Q07898|Q07898
tr|Q07901|Q07901
tr|Q07900|Q07900
tr|Q07899|Q07899

"Functional equivalency" as used in the present invention is according to one preferred embodiment established by means of reference to the corresponding functionality of a predetermined CD163 fragment.

According to the present invention a functional equivalent of a CD163 variant or fragments thereof may be obtained by addition, substitution or deletion of at least

one amino acid. When the amino acid sequence comprises a substitution of one amino acid for another, such a substitution may be a conservative amino acid substitution. Fragments of CD163 according to the present invention may comprise more than one such substitution, such as e.g. two conservative amino acid substitutions, for example three or four conservative amino acid substitutions, such as five or six conservative amino acid substitutions, for example seven or eight conservative amino acid substitutions, such as from 10 to 15 conservative amino acid substitutions, for example from 15 to 25 conservative amino acid substitution. Substitutions can be made within any one or more groups of predetermined amino acids.

Examples of fragments comprising one or more conservative amino acid substitutions including one or more conservative amino acid substitutions within the same group of predetermined amino acids, or a plurality of conservative amino acid substitutions, wherein each conservative substitution is generated by substitution within a different group of predetermined amino acids.

Accordingly, variant of CD163, or fragments thereof according to the invention may comprise, within the same variant of CD163, or fragments thereof, at least one substitution, such as a plurality of substitutions introduced independently of one another. Variants of CD163, or fragments thereof may thus comprise conservative substitutions independently of one another, wherein at least one glycine (Gly) of said variants of CD163, or fragments thereof of CD163 is substituted with an amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and Ile, and independently thereof, variant of CD163, or fragments thereof, wherein at least one of said alanines (Ala) of said variant of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Val, Leu, and Ile, and independently thereof, variant of CD163, or fragments thereof, wherein at least one valine (Val) of said variant of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Leu, and Ile, and independently thereof, variants of CD163, or fragments thereof, wherein at least one of said leucines (Leu) of said variant of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and Ile, and independently thereof, variants of CD163, or fragments thereof, wherein at least one isoleucine (Ile) of said variants of CD163, or fragments thereof is substituted with an amino acid selected from the group of

amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, variants of CD163, or fragments thereof wherein at least one of said aspartic acids (Asp) of said variants of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Glu, Asn, and Gln, and independently thereof, variants of CD163, or fragments thereof, wherein at least one of said phenylalanines (Phe) of said variants of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, variants of CD163, or fragments thereof, wherein at least one of said tyrosines (Tyr) of said variants of CD163, or fragments thereof of CD163 is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, variants of CD163, or fragments thereof, wherein at least one of said arginines (Arg) of said fragment of CD163 is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, variants of CD163, or fragments thereof, wherein at least one lysine (Lys) of said variants of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, variants of CD163, or fragments thereof, wherein at least one of said aspargines (Asn) of said variants of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, variants of CD163, or fragments thereof, wherein at least one glutamine (Gln) of said variants of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and independently thereof, variants of CD163, or fragments thereof, wherein at least one proline (Pro) of said variants of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, variants of CD163, or fragments thereof, wherein at least one of said cysteines (Cys) of said variants of CD163, or fragments thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

It is clear from the above outline that the same variant or fragment thereof may comprise more than one conservative amino acid substitution from more than one group of conservative amino acids as defined herein above.

Conservative substitutions may be introduced in any position of a preferred predetermined CD163 variant of fragment thereof. It may however also be desirable to introduce non-conservative substitutions, particularly, but not limited to, a non-conservative substitution in any one or more positions.

A non-conservative substitution leading to the formation of a functionally equivalent fragment of CD163 would for example i) differ substantially in hydrophobicity, for example a hydrophobic residue (Val, Ile, Leu, Phe or Met) substituted for a hydrophilic residue such as Arg, Lys, Trp or Asn, or a hydrophilic residue such as Thr, Ser, His, Gln, Asn, Lys, Asp, Glu or Trp substituted for a hydrophobic residue; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

Substitution of amino acids may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like. Exemplary amino acid substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

The addition or deletion of an amino acid may be an addition or deletion of from 2 to preferably 10 amino acids, such as from 2 to 8 amino acids, for example from 2 to 6 amino acids, such as from 2 to 4 amino acids. However, additions of more than 10 amino acids, such as additions from 10 to 200 amino acids, are also comprised within the present invention.

It will thus be understood that the invention concerns CD163 variants comprising at least one fragment of CD163 capable of binding at least one Hp-Hb complex, including any variants and functional equivalents of such at least one fragment.

The CD163 variant according to the present invention, including any functional equivalents and fragments thereof, may in one embodiment comprise less than 1000 amino acid residues, such as less than 950 amino acid residues, for example less than 900 amino acid residues, such as less than 850 amino acid residues, for example less than 800 amino acid residues, such as less than 750 amino acid residues, for example less than 700 amino acid residues, such as less than 650 amino acid residues, for example less than 600 amino acid residues, such as less than 550 amino acid residues, for example less than 500 amino acid residues, such as less than 450 amino acid residues, for example less than 400 amino acid residues, such as less than 380 amino acid residues, for example less than 370 amino acid residues, such as less than 360 amino acid residues, for example less than 350 amino acid residues, such as less than 340 amino acid residues, for example less than 330 amino acid residues, such as less than 320 amino acid residues, for example less than 310 amino acid residues, such as about 300 amino acid residues, for example less than 300 amino acid residues, such as about 290 amino acid residues, for example 290 amino acid residues.

A fragment comprising the Hp-Hb binding region of native CD163 is particularly preferred. However, the invention is not limited to fragments comprising the Hp-Hb binding region. Deletions of such fragments generating functionally equivalent fragments of CD163 comprising less than the Hp-Hb binding region are also comprised in the present invention. Functionally equivalent CD163 peptides, and fragments thereof according to the present invention, may comprise less or more amino acid residues than the Hp-Hb binding region.

Fragments comprising the Hp-Hb binding region preferably comprises the SRCR domains I-IX of the CD163 receptor, such as capable of binding to a region in the SRCR domains I-VIII of the CD163 receptor, capable of binding to a region in the SRCR domains I-VII of the CD163 receptor, capable of binding to a region in the SRCR domains I-VI of the CD163 receptor, capable of binding to a region in the SRCR domains I-V of the CD163 receptor, capable of binding to a region in the

SRCR domains I-IV of the CD163 receptor, capable of binding to a region in the SRCR domains I-III of the CD163 receptor, capable of binding to a region in the SRCR domains I-II of the CD163 receptor, or a variant thereof.

Functional equivalents of variants of CD163 will be understood to exhibit amino acid sequences gradually differing from the preferred predetermined sequence, as the number and scope of insertions, deletions and substitutions including conservative substitutions increases. This difference is measured as a reduction in homology and/or identify between the preferred predetermined sequence and the fragment or functional equivalent.

All fragments or functional equivalents of CD163 variants are included within the scope of this invention, regardless of the degree of homology that they show to a preferred predetermined sequence of CD163 variants. The reason for this is that some regions of CD163 are most likely readily mutatable, or capable of being completely deleted, without any significant effect on the binding activity of the resulting fragment.

A functional variant obtained by substitution may well exhibit some form or degree of native CD163 activity, and yet be less homologous, if residues containing functionally similar amino acid side chains are substituted. Functionally similar in this respect refers to dominant characteristics of the side chains such as hydrophobic, basic, neutral or acidic, or the presence or absence of steric bulk. Accordingly, in one embodiment of the invention, the degree of identity between i) a given CD163 fragment capable of effect and ii) a preferred predetermined fragment, is not a principal measure of the fragment as a variant or functional equivalent of a preferred predetermined CD163 fragment according to the present invention.

Fragments sharing at least some homology with a preferred predetermined CD163 fragment of at 50 amino acids, preferably at least 100 amino acids, are to be considered as falling within the scope of the present invention when they are at least about 40 percent homologous with the predetermined CD163 variant or fragment thereof, such as at least about 50 percent homologous, for example at least about 60 percent homologous, such as at least about 70 percent homologous, for example at least about 75 percent homologous, such as at least about 80 percent homolo-

gous, for example at least about 85 percent homologous, such as at least about 90 percent homologous, for example at least 92 percent homologous, such as at least 94 percent homologous, for example at least 95 percent homologous, such as at least 96 percent homologous, for example at least 97 percent homologous, such as at least 98 percent homologous, for example at least 99 percent homologous homologous with the predetermined CD163 fragment. In a preferred embodiment the percentages mentioned above also relates to identify percentages.

In addition to the variants described herein, sterically similar variants may be formulated to mimic the key portions of the variant structure and that such compounds may also be used in the same manner as the variants of the invention. This may be achieved by techniques of modelling and chemical designing known to those of skill in the art. It will be understood that all such sterically similar constructs fall within the scope of the present invention.

In one embodiment the CD163 variant is synthesised by automated synthesis. Any of the commercially available solid-phase techniques may be employed, such as the Merrifield solid phase synthesis method, in which amino acids are sequentially added to a growing amino acid chain. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied Biosystems, Inc. of Foster City, Calif., and may generally be operated according to the manufacturer's instructions. Solid phase synthesis will enable the incorporation of desirable amino acid substitutions into any CD163 variant according to the present invention. It will be understood that substitutions, deletions, insertions or any subcombination thereof may be combined to arrive at a final sequence of a functional equivalent. Insertions shall be understood to include amino-terminal and/or carboxyl-terminal fusions, e.g. with a hydrophobic or immunogenic protein or a carrier such as any polypeptide or scaffold structure capable as serving as a carrier.

CD163 variants according to the invention may be synthesised both in vitro and in vivo. Method for in vitro synthesis are well known. When synthesized in vivo, a host cell is transformed with vectors containing DNA encoding the CD163 variant. A vector is defined as a replicable nucleic acid construct. Vectors are used to mediate expression of the CD163 variant. An expression vector is a replicable DNA construct in which a nucleic acid sequence encoding the predetermined CD163 variant, or any

functional equivalent thereof that can be expressed in vivo, is perably linked to suitable control sequences capable of effecting the expression of the variant, or equivalent in a suitable host. Such control sequences are well known in the art.

Accordingly, one aspect of the invention relates to a DNA sequence encoding a CD163 variant as defined above, the DNA sequence may be a genomic DNA sequence, a cDNA sequence or a mixture of a genomic and a cDNA sequence.

Furthermore, the invention relates to a vector comprising the DNA sequence, as well as to a cell comprising said vector, said cell being capable of expressing the DNA sequence, either as a CD163 variant released into the cell culturing media, or a CD163 variant anchored to the cell membrane.

Cultures of cells derived from multicellular organisms represent preferred host cells. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of useful host cell lines are E-coli, yeast or human cell lines. Preferred host cells are eukaryotic cells known to synthesize endogenous CD163. Cultures of such host cells may be isolated and used as a source of the variant, or used in therapeutic methods of treatment, including therapeutic methods aimed at diagnostic methods carried out on the human or animal body.

Multimers and dimers, including homodimers and heterodimers of variants of CD163 according to the invention, are also provided and fall under the scope of the invention. CD163 functional equivalents and fragments can be produced as homodimers or heterodimers with other amino acid sequences or with native CD163 sequences. Heterodimers include dimers containing a CD163 variant binding at least one Hp-Hb complex when present in a homodimer, and a CD163 fragment that need not have or exert any biologically activity.

The binding affinity of the CD163 variant of the invention and a dimeric Hp-Hb complex preferably has a kD value of between 10-100 nM, such as between 20-80 nM, for example between 40-60 nM, such as between 45-55 nM.

The CD163 variant of the invention preferably has a Kd binding affinity for a multimeric Hp-Hb complex of the invention of between 2-10 nM.

A dimeric Hp-Hb complex preferably has a binding affinity to two CD163 receptors on a cell in the range of from 0.05 to 1.0 nM.

The binding affinity may be determined as discussed in Example 2 and 3 below.

One aspect of the invention relates to a composition comprising at least one purified CD163 receptor and/or at least one purified CD163 receptor variant as defined above.

Another aspect of the invention relates to a composition comprising a Hp-Hb complex or a part thereof or a mimic thereof as defined above.

The composition(s) is(are) particularly useful in the manufacture of a medicament for any of the uses discussed below.

The medicament is preferably suitable for parenteral administration, such as intravenous, intramuscular, subcutaneous, or intravenous administration. Thus, the medicament may further comprise any suitable carriers, adjuvants, and/or additives conventionally used for the preparation of medicaments, in particular medicaments for parenteral administration. Another suitable administration route is via inhalation.

The present invention further relates to the following applications of Hp-Hb complexes and/or a variant thereof. One such use is in the manufacture of a medicament for treatment of conditions related to haemolysis in an individual in need of such treatment. Another such use of at least one CD163 or a variant thereof is for the removal of at least one Hp-Hb complex in serum and/or plasma of an individual. The invention may also be used for the determination of the haemolysis rate of an individual. This may be done by determining the level of the binding activity between the CD163 variant and the Hp-Hb complexes, as an indication of the rate with which red blood cells are lysed.

The invention also relates to the use of at least one CD163 molecule for the identification of at least one Hp-Hb complex in serum and/or plasma of an individual.

In yet another aspect the invention relates to the uses of at least ne complex comprising haemoglobin and haptoglobin. For example the complex may be used as a marker for a cell expressing CD163 or a CD163 variant, wherein at least one of the haemoglobin or haptoglobin molecules are labelled. Such cell may be a macrophage. Another use is for the delivery of at least one drug/medicament or at least one gene to a cell to a cell expressing CD163 or a CD163 variant. The processes of drug and gene-delivery are mentioned above.

The purpose of drug or gene delivery is to localize the drug to the target site. Such targeted delivery systems often take the form of injectables composed of liposomes and microspheres made of proteins. Polymeric systems share some of the advantages of liposomal systems such as altered pharmacokinetics and biodistribution. While liposomes might have better prospects of biocompatibility and potential for fusion with cells, polymeric microspheres have more controllable release kinetics, better stability in storage, and higher drug-loading levels for some classes of compounds. The delivery system is targetted through a linkage to at least one Hp-Hb complex capable of binding to CD163 or a variant thereof.

The delivery may made in vivo or in vitro, the latter in particular being for experimental purposes.

In particular the drugs and genes delivered may be selected from the medicaments discussed above.

The deliberate introduction of DNA encoding a desired gene, under conditions where the gene may be expressed within the cell and leads to the production of RNA and/or protein, can be desirable in order to provoke any of a wide range of useful biological responses. The Hp-Hb complex can carry heterologous genes under the control of promoters able to cause their expression in vectors.

In another aspect of the invention the gene therapy comprises introducing a nucleic acid sequence to up-regulate or down-regulate expression of a target gene in the host cell, either by means of a protein encoded by the introduced nucleic acid sequence or by means of an anti-sense relation between RNA encoded by the introduced nucleic acid and a target nucleic acid molecule corresponding to an endogenous gene product.

It is a further object of the present invention that the CD163 or CD163 variant is applied in a method comprising the treatment of haemolysis in an individual in need of such treatment. Lysis of red blood cells may occur in a number of physiological and pathological conditions. The release of haemoglobin to the plasma presents a serious physiological threat. Administration of CD163 or the CD163 variant leads to a binding between the Hp-Hb complexes formed due the haemolysis and CD163, whereby fewer Hp-Hb complexes are taken up by the macrophages leading to a less severe hemosiderosis.

In another embodiment the same effect may be obtained by administrating antibodies directed to the CD163 receptor. The antibodies may be monoclonal, such as those mentioned below in the examples or polyclonal. Production of antibodies is known to the skilled person.

In a further embodiment Hp-Hb complexes are administered to inhibit uptake of native Hp-Hb complexes again leading to a less severe hemosiderosis.

In yet a further aspect of the invention the CD163 variant is used in a method for the removal of at least one Hp-Hb complex in serum and/or plasma of an individual. Since the present inventors have now established CD163 and CD163 variants as the acute phase-regulated capture protein for Hp-Hb complexes the CD163 variant may be applied to an individual in need of plasma haemaglobin clearance.

This may also be accomplished by gene therapy, by administration of genes encoding CD163 or a variant thereof, in order to produce cells capable of assisting the macrophages in case of plasma haemoglobin clearance.

In another embodiment of the invention the CD 163 variant is used in a diagnostic method. One such diagnostic method is for marking a cell expressing a CD163 variant, wherein at least one of the haemoglobin or haptoglobin molecules or parts thereof are labelled. It is possible to identify CD163 variants *in vitro* as well as *in vivo* by bringing into contact at least one Hp-Hb complex with an environment comprising CD163 variants. The individual haemoglobin or haptoglobin molecules may be labelled with a marker as discussed above. In one aspect of the invention the CD163

variant is used in a diagnostic method for identifying monocytes and/or macrophages in an individual or in vitro.

In another aspect the CD163 variant is used in a method for the identification of at least one Hp-Hb complex in serum and/or plasma of an individual.

In this aspect the CD163 variant may be used for determination of the haemolysis rate of an individual.

Furthermore, the Hp-Hb complex linked to a marker may be used for identification of monocytes, such as macrophages, in tissues, such as sections of tissues for example for microscopic examinations.

In another embodiment the Hp-Hb complex linked to a marker may be used for detection of CD163, either membrane bound CD163 and/or soluble CD 163. In particular the Hp-Hb complex linked to a marker may be used for detection soluble CD 163 in a sample, such as a blood sample.

Experimentals

Example 1

Purification and identification of the Hp-Hb receptor.

Human Hp (1-1, 2-2, and mixed phenotypes) and human Hb (A_0, A_2 and S forms) were from Sigma. A five ml Hp-Hb Sepharose CL-4B (Pharmacia-Amersham) column was prepared by coupling complexes of Hp (5 mg, mixed phenotypes) and Hb (4 mg, type A_0). The column was loaded with 100 ml ~1% Triton X-100 solubilised membranes (from human spleen, placenta, and liver), prepared as previously described (Moestrup, S. K., Kaltoft, K., Sottrup-Jensen, L. & Gliemann, J. The human α_2-macroglobulin receptor contains high affinity calcium binding sites important for receptor conformation and ligand recognition. *J. Biol. Chem.* **265**, 12623-12628 (1990).. The purified 130 kDa protein binding Hp-Hb was eluted in 10 mM NaH_2PO_4 (pH 6), 150 mM NaCl, 5 mM EDTA and 0.5% CHAPS (Aldrich). SDS-gel separated protein was processed for tryptic digestion and MALDI mass spectrometry by Protana (Odense, Denmark). The difference in calculated and measured masses was for all peptides less than 0.042 kDa. The murine monoclonal CD163 antibodies EDHu-1 (Serotec) and GHI/61 (Research Diagnostics) were used for western blotting. A polyclonal CD163 antibody was raised by immunisation of a rabbit with ligand-affinity purified receptor.

Example 2

Ligand-receptor binding analysis

Surface plasmon resonance analysis was carried out as described Moestrup, S. K. *et al.* β_2-glycoprotein-I (apolipoprotein H) and β_2-glycoprotein-I- phospholipid complex harbor a recognition site for the endocytic receptor megalin. *J. Clin. Invest* **102**, 902-909 (1998). Purified CD163 was immobilised at the BIAcore sensor CM5 chip (BIAcore AB) at a concentration of up to 50 µg/ml in 10 mM sodium acetate, pH 4.0, and the remaining binding sites were blocked with 1 M ethanolamine pH 8.5. The surface plasmon resonance signal generated from immobilised CD163 corresponded to 55 – 66 fmol receptor/mm^2. The sample and flow buffer was 10 mM

Hepes, 150 mM NaCl, 0.5 mM $CaCl_2$, pH 7.4. The sensor chips were regenerated with 1.6 M glycine-HCl, pH 3. The binding assay for measuring binding of ^{125}I-Hp-Hb to human CD163 immobilised in microtiter plate wells (Nunc) was carried out as described Birn, H. *et al.* Characterization of an epithelial approximately 460-kDa protein that facilitates endocytosis of intrinsic factor-vitamin B12 and binds receptor-associated protein. *J. Biol. Chem.* **272**, 26497-26504 (1997).

The microtiter plates were coated at 4°C for 20 h with purified CD163 in 50 mM $NaHCO_3$ containing 250 ng CD163 per well (for binding ^{125}I-Hp(1-1)-Hb) or 125 ng CD163 per well (for binding ^{125}I-Hp(2-2)-Hb). Iodination of Hp-Hb was performed with the chloramine-T-method. Ligand blotting was carried out as described using 10^6 cpm radioligand/ml (Moestrup, S. K. & Gliemann, J. Analysis of ligand recognition by the purified α_2-macroglobulin receptor (low density lipoprotein receptor-related protein). Evidence that high affinity of α_2-macroglobulin-proteinase complex is achieved by binding to adjacent receptors. *J. Biol. Chem.* **266**, 14011-14017 (1991).α

Hp is synthesised as a single chain, which is post-translationally cleaved into an amino-terminal α chain and a carboxy-terminal β chain. The basic structure of Hp, as found in most mammals, is a homodimer (Fig. 2a), in which the two Hp molecules are linked by a single disulfide bond via their respective ~9 kDa α chains[14]. In man, a variant with a long α chain is also present in all populations. This variant arose apparently by an early intragenic duplication, presumably originating from an unequal crossover of two basic alleles, resulting in an Hp with an α chain of ~14 kDa. The short and long α chains are designated as α^1 and α^2, respectively. Since the cysteine forming the intermolecular disulfide bond between the α chains is also duplicated, humans carrying the long variant allele exhibit a multimeric Hp phenotype (Fig. 2a).

Analysis of the binding of Hp-Hb complexes (Fig. 2a) to immobilised CD163 revealed a high-affinity binding of both dimeric and multimeric Hp-Hb complexes (Fig. 2b and c). Fig. 2b shows a surface plasmon resonance analysis of CD163 binding of the dimeric Hp(1-1)-Hb complex and the multimeric Hp(2-2)-Hb complex. No binding of non-complexed Hb (Fig. 2b, left panel) nor Hp(1-1) or Hp(2-2) (Fig 2b, middle and right panels) was detected thus indicating that a neoepitope for receptor

binding is expressed in the Hp-Hb complex. Accordingly, maximal receptor binding was measured, when the Hb binding capacity of Hp reached saturation (Fig. 2b, middle and right panels) at equimolar concentrations of Hb and Hp. The Hp(2-2)-Hb complex yielded a higher response and the dissociation was slower as compared to the Hp(1-1)-Hb complex. The results shown were obtained using the A_0 ($\alpha_2\beta_2$) form of Hb. Similar results were obtained using the A_2 ($\alpha_2\delta_2$) form or the S form (Hb with the mutation for sickle cell disease) [15] (data not shown).

Example 3

Binding affinity

A solid phase assay with immobilised CD163 in microtiter wells was used for various inhibition experiments (Fig. 6c). This analysis revealed that the removal of Ca^{2+} with EDTA or the addition of polyclonal anti-CD163 IgG completely abolished the binding of Hp-Hb to CD163. Measuring the true affinity of the one-site interaction of Hp-Hb binding to CD163 was hampered by the suggested divalency (Hp(1-1)) and multivalency (Hp(2-2)) of the ligand in terms of receptor-recognition sites. However, competition for CD163-binding of ^{125}I-labelled Hp-Hb by unlabelled Hp(1-1)-Hb and Hp(2-2)-Hb complexes showed, as anticipated from the surface plasmon resonance experiments, an ~10 fold higher functional affinity (avidity) of the multimeric Hp(2-2)-Hb complexes (Fig. 6c). The concentration of unlabelled Hp(1-1)-Hb complex causing 50% inhibition of the binding of ^{125}I-labelled Hp(1-1)-Hb was ~0.3 µg/ml, giving an 'apparent K_d' of ~2 nM of the dimeric Hp(1-1)-Hb complex. In contrast, the 50% inhibition point for Hp(2-2)-Hb was at ~0.1 µg/ml giving an 'apparent K_d' of ~0.2 nM (on assumption of the 2-2 multimer distribution previously calculated Wejman, J. C., Hovsepian, D., Wall, J. S., Hainfeld, J. F. & Greer, J. Structure and assembly of haptoglobin polymers by electron microscopy. *J. Mol. Biol.* **174**, 343-368 (1984).). The higher functional affinity of the 2-2 type complex is probably accounted for by its higher valency. Similar 'bonus effect of multivalency' is well known in other biological systems, e.g. the binding of the pentameric IgM molecule to several identical surface antigens.

Example 4

Endocytosis analysis in CD163-transfected CHO cells and in SU-DHL cells

The cDNA encoding the most abundant variant of CD163 (Genbank/EMBL accession no Z22968) Law, S. K. *et al.* A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. *Eur. J. Immunol.* **23**, 2320-2325 (1993) was ligated into the KpnI and NotI sites of the mammalian expression vector pcDNA3.1/Zeo(+) (Invitrogen). Stable transfected CHO clones expressing CD163 were established by limited dilution and selection with 500 μg/ml Zeocin (Invitrogen). Expression products were analysed by immunoblotting of growth medium and cell lysate using the rabbit polyclonal antibody against the ligand-affinity purified human CD163.

Endocytosis of ^{125}I-Hp-Hb in CD163-tranfected and mock-transfected CHO cells growing as confluent adherent monolayers in 24-well plates was analysed as previously described Moestrup, S. K. & Gliemann, J. Analysis of ligand recognition by the purified α_2-macroglobulin receptor (low density lipoprotein receptor-related protein). Evidence that high affinity of α_2-macroglobulin-proteinase complex is achieved by binding to adjacent receptors. *J. Biol. Chem.* **266**, 14011-14017 (1991). Endocytosis in the soluble SU-DHL-1 histiocytic lymphoma cells (2×10^6 cell/ml) was analysed as described Moestrup, S. K., Christensen, E. I., Sottrup-Jensen, L. & Gliemann, J. Binding and receptor-mediated endocytosis of pregnancy zone protein-proteinase complex in rat macrophages. *Biochim. Biophys. Acta* **930**, 297-303 (1987).

CD163-mediated endocytosis of ^{125}I-Hp-Hb complexes was studied in Chinese Hamster Ovary (CHO) cells transfected with CD163 cDNA (the abundant CD163 form, Genbank/EMBL accession no Z22968). Fig. 7a (middle panel) shows the time course of cell-associated radioactivity and trichloroacetic acid (TCA)-soluble radioactivity (representing degraded ligand) in the medium. The cell-associated radioactivity reached a plateau after one hour of incubation, and about this time, the TCA-soluble radioactivity significantly increased in the medium. Consistent with an endocytic uptake of Hp-Hb, a similar experiment conducted in the presence of the lysosomal inhibitors, chloroquine and leupeptin, showed a continual increase in cell-

bound radioactivity for 3 hours with essentially no TCA-soluble radioactivity detected (Fig. 7a, right panel).

The endocytosis of Hp-Hb complexes was mediated by CD163, since no uptake, and consequently no TCA-soluble radioactivity, was detected in incubations with CHO cells not expressing the CD163 antigen (Fig. 7a, left panel). Furthermore, uptake and degradation of ^{125}I-labelled Hp(2-2)-Hb can be inhibited by purified IgG from anti-CD163 serum and by unlabelled Hp(2-2)-Hb complexes (Fig. 7b, left panel). Similar results (Figure 7b, right panel) were obtained with the myelomonocytic SU-DHL-1 cell line (Epstein, A. L. *et al.* Biology of the human malignant lymphomas. IV. Functional characterization of ten diffuse histiocytic lymphoma cell lines. *Cancer* **42**, 2379-2391 (1978), the only cell line Pulford, K., Micklem, K., Law, S. K. & Mason, D. Y. in Leukocyte Typing VI. (eds. Kishimoto, T. *et al.*) 1089-1091 (Garland Publishing Inc, New York, 1997) known to express the CD163 antigen, and with ^{125}I-labelled Hp(1-1)-Hb complexes although a lower rate of uptake was observed in comparison with the ^{125}I-labelled Hp(2-2)-Hb complexes (data not shown). The SU-DHL cell line expresses, in addition to the most abundant CD163 variant, also two less abundant variants Law, S. K. *et al.* A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. *Eur. J. Immunol.* **23**, 2320-2325 (1993) with different cytoplasmic tails.

Claims

1. A Hp-Hb complex (haptoglobin-haemoglobin complex) or a part thereof or a mimic thereof being operably linked to a substance, wherein the Hp-Hb complex or the part thereof or the mimic thereof is capable of binding a CD163 receptor and/or a CD163 receptor variant.

2. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 1, in a purified form.

3. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 1, in an isolated form.

4. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 1, wherein the complex is dimeric.

5. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 1, wherein the complex is multimeric.

6. The Hp-Hb part or mimic according to claim 1, capable of binding to a region in the SRCR domains I-IX of the CD163 receptor.

7. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 1, wherein the substance comprises a medicament.

8. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 7, wherein the medicament is selected from antibiotics and anticancer drugs.

9. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 8, wherein the medicament is selected from anti-HIV drugs.

10. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 1, wherein the substance comprises a gene.

11. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 10, wherein the gene comprises a nucleic acid, such as PNA, LNA, DNA or RNA.

12. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 10, wherein the gene comprises cDNA.

13. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 10, wherein the gene is encoding CD163.

14. The Hp-Hb complex or a part thereof or a mimic thereof according to claim 1, wherein the substance comprises an antibody or an antigen.

15. The Hp-Hb complex or a part thereof or a mimic thereof according to any of the claims 1-14, wherein the substance comprises a tracer or a marker.

16. The Hp-Hb complex or a part thereof or a mimic thereof according to any of claims 1-15 for use as a medicament.

17. A method of treatment of an individual comprising administration of the Hp-Hb complex or a part thereof or a mimic thereof according to any of claims 1-14, the method comprising the steps of:

iv) providing a Hp-Hb complex, or a part thereof, as defined in any of the claims 1-16 capable of binding to the CD163 variant,

v) operably linking a substance as defined in the claims 1-16 to the Hp-Hb complex, or a part thereof or a mimic thereof,

vi) administering the substance operably linked to the Hp-Hb complex or a part thereof or a mimic thereof to an individual in need thereof.

18. A composition comprising a Hp-Hb complex or a part thereof or a mimic thereof as defined in any of claims 1-17.

19. A CD163 variant capable of binding at least one haptoglobin-haemoglobin (Hp-Hb) complex.

20. The CD163 variant according to claim 19, wherein the at least one Hp-Hb complex is dimeric.

21. The CD163 variant according to claim 19, wherein the at least one Hp-Hb complex is multimeric.

22. The CD163 variant according to claim 19, comprising the SRCR domains I-IX of the CD 163 receptor or a variant thereof

23. The CD163 variant according to claim 22, comprising at least the Hp-Hb binding region.

24. The CD163 variant according to claim 19, wherein the binding affinity has a Kd of 100 nM or less.

25. The CD163 variant according to claim 19, wherein the binding affinity has a Kd of 10 nM or less.

26. The CD163 variant according to any of claims 18 to 25 for use as a medicament.

27. A method of treatment of an individual comprising administration of a CD163 variant according to any of claims 18 to 25.

28. The method according to claim 17, wherein the method comprises drug-delivery treatment of an individual in need of such treatment.

29. The method according to claim 17, wherein the method comprises gene delivery treatment of an individual in need of such treatment.

30. The method according to claim 17 or 27, wherein the method comprises treatment of haemolysis in an individual in need of such treatment.

31. The CD 163 variant according to any of claims 19 t 26 for use in a diagnostic method.

32. The CD163 variant according to claim 31 said CD163 being expressed by a cell said method comprising administrating a Hp-Hb complex operably linked to a marker for marking the CD163 representing cell.

33. The CD163 variant according to claim 32 for identifying macrophages in a biological sample from an individual.

34. The CD163 variant according to claim 19-26, wherein the method is for the identification of at least one Hp-Hb complex in serum and/or plasma of an individual.

35. The CD163 variant according to any of the claims 19-26, wherein the method is for the removal of at least one Hp-Hb complex in serum and/or plasma of an individual.

36. A composition comprising at least one purified CD163 receptor and/or at least one purified CD163 variant according to any of the claims 19-26.

37. Use of a composition as defined in claim 36 in the manufacture of a medicament.

38. The use according to claim 37, wherein the medicament is for treatment of haemolysis in an individual in need of such treatment.

39. The use according to claim 37 wherein the medicament is for the removal of at least one Hp-Hb complex in serum and/or plasma of an individual.

40. The use according to claim 37, wherein the medicament is for determination of the haemolysis rate of an individual.

41. Use of a composition as defined in claim 18 in the manufacture of a medicament.

42. The use according to claim 41, wherein the medicament is a marker for a cell expressing a CD163 variant according to the claims 19-35, wherein at least one of the haemoglobin or haptoglobin molecules are labelled.

43. The use according to claim 42, wherein the cell is a macrophage.

44. The use according to claim 41, wherein the medicament comprises at least one drug to be delivered to a cell expressing a CD163 receptor or a CD163 variant as defined in any of the claims 19-26.

45. The use according to claim 41, wherein the medicament comprises at least one gene to be delivered a cell expressing a CD163 receptor or a CD163 variant as defined in any of the claims 19-26.

46. The use according to claim 44, wherein the medicament comprises an antibiotic or an anticancer drug.

47. The use according to claim 45, wherein the medicament comprises an anti HIV drug.

48. The use according to claim 41, wherein the medicament further comprises a CD163 variant as defined in any of the claims 19-26 for the identification of at least one Hp-Hb complex in serum and/or plasma of an individual.

49. Use of at least one complex comprising haemoglobin and haptoglobin as a marker for a cell expressing a CD163 molecule as defined in any of claims19-26, wherein at least one of the haemoglobin or haptoglobin molecules are labelled.

50. The use according to claim 49, wherein the cell is a macrophage.

51. Use of at least one Hp-Hb complex for the delivery of at least one drug to a cell expressing a CD163 molecule as defined in claims 19-26.

52. Use of at least one Hp-Hb complex for the delivery of at least one gene to a cell expressing a CD163 molecule as defined in claims 19-26.

53. A DNA sequence encoding a CD163 variant as defined in any of claims 19-26.

54. A DNA sequence encoding at least one polypeptide capable of forming a Hp-Hb complex, said complex capable of binding to a CD163 receptor anchored to a cell, whereby a substance operably linked to said polypeptide is taken up by the cell.

55. A vector comprising a DNA sequence as defined in claim 55 or claim 56.

56. A cell comprising a vector as defined in claim 57.

Figure 1

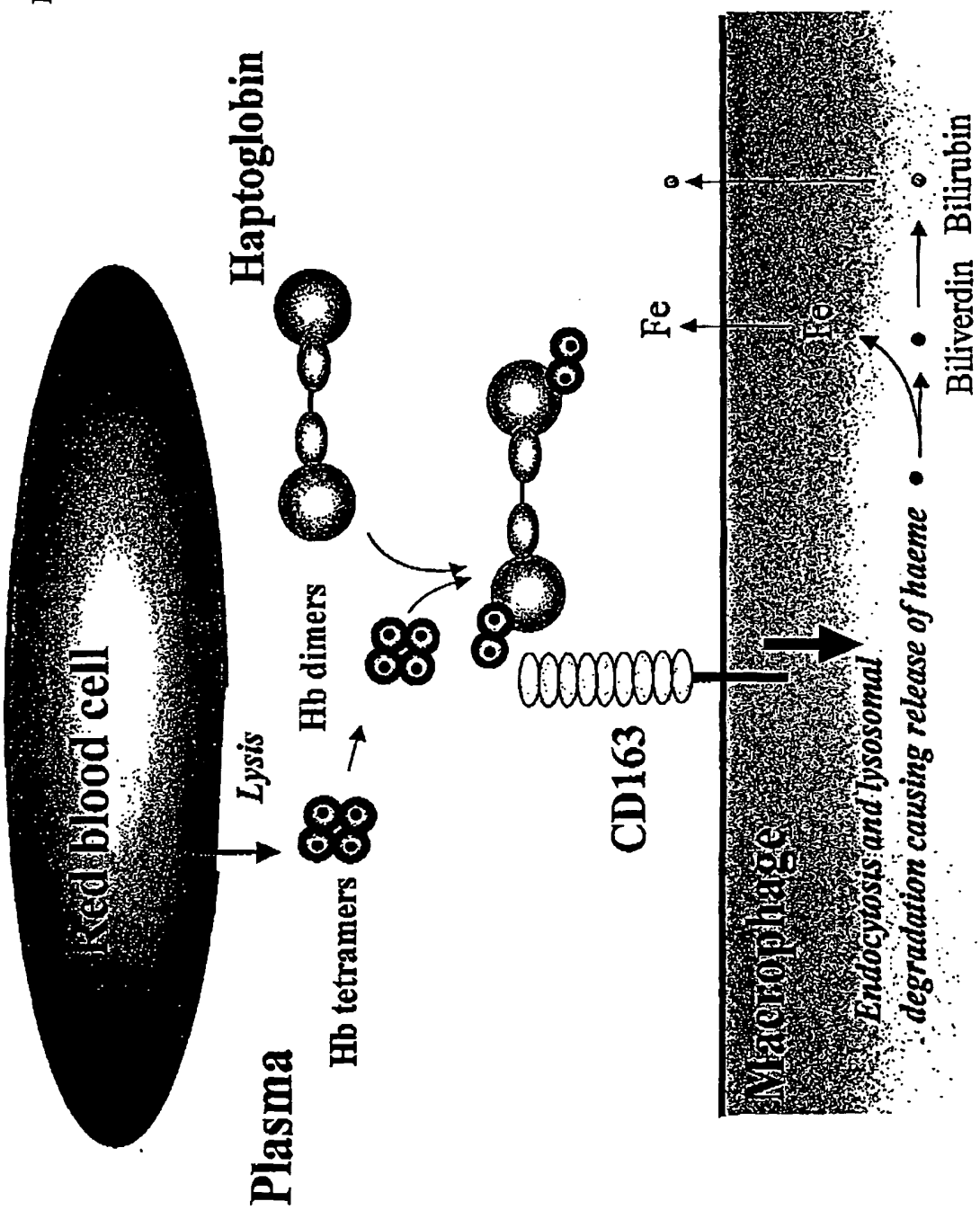

Figure 2

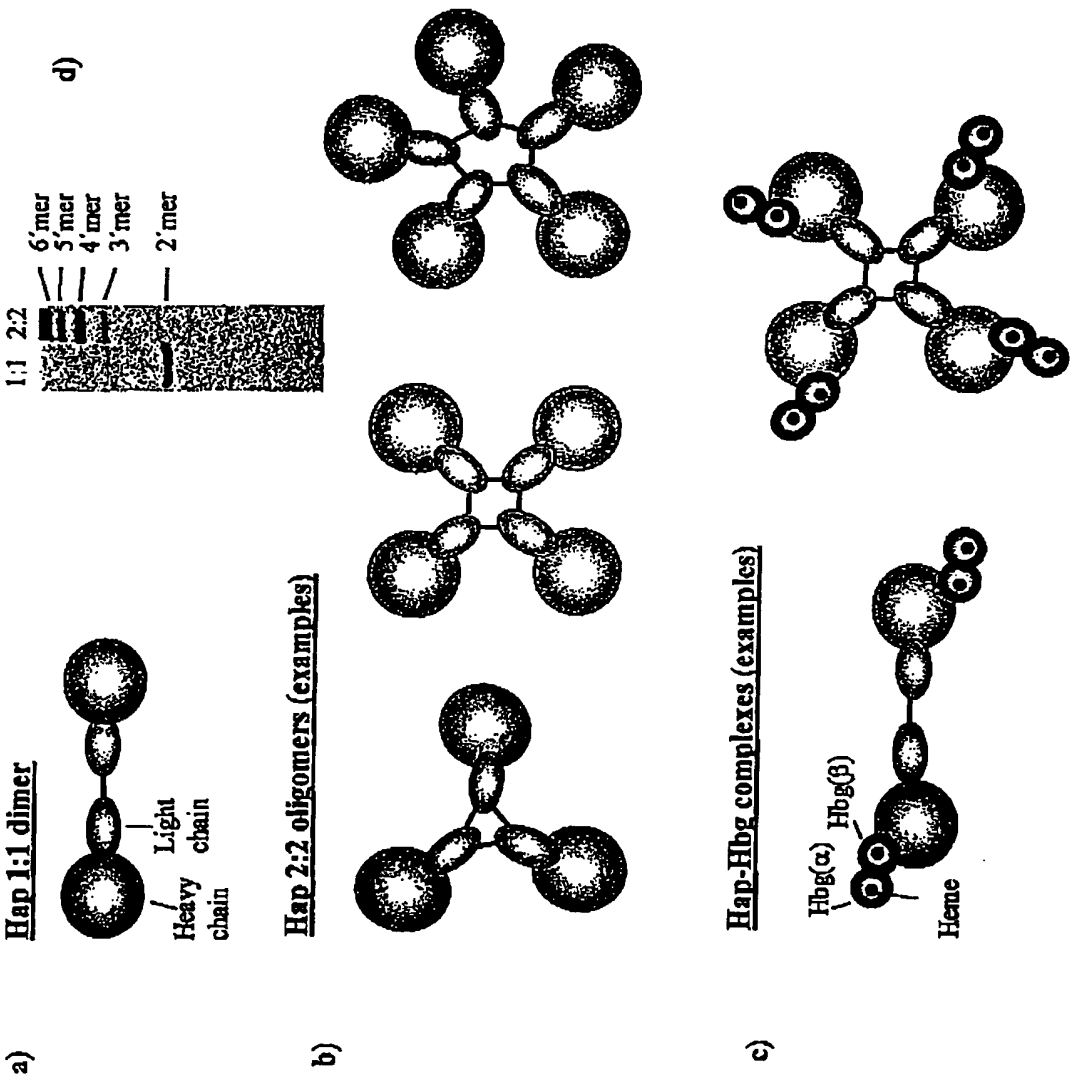

Figure 3

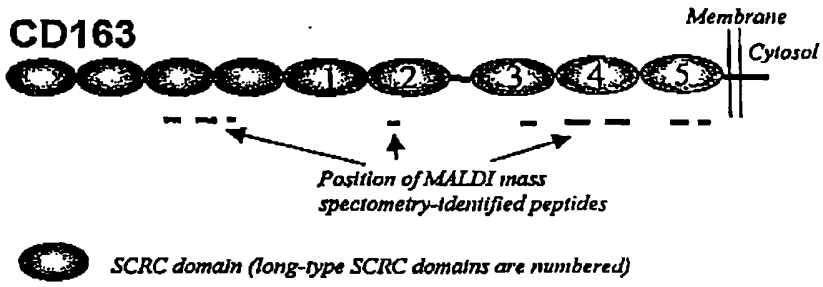

Figure 4a

```
sp|P00737|HPT1_HUMAN      MSALGAVIALLLWGQLFAVDSGNDVTDIADDGCPKPPEIANGYVEHSVRYQCKNYYKLRT 60
sp|P00738|HPT2_HUMAN      MSALGAVIALLLWGQLFAVDSGNDVTDIADDGCPKPPEIANGYVEHSVRYQCKNYYKLRT 60
sp|P50417|HPT_ATEGE       MSALGAVIALLLWGQLFAVDSGNDVTDIADDGCPKPPSIANGYVEHLVRYQCKKYYRLRT 60
tr|Q60574|Q60574          MRALGAVVTLLLWGQLFAVELGNDAMDFEDDSCPKPPEIANGYVEHLVRYRCRQFYRLRA 60
tr|Q61646|Q61646          MRALGAVVTLLLWGQLFAVELGNDAMDFEDDSCPKPPEIANGYVEHLVRYRCRQFYRLRA 60
sp|Q62558|HPT_MUSSA       MRALGAVVTLLLWGQLFAAELGNDAMDFEDDSCPKPPEIANGYVEHLVRYRCRQFYRLRT 60
sp|P06866|HPT_RAT         MRALGAVVTLLLWGQLFAVELGNDATDIEDDSCPKPPEIANGYVEHLVRYRCRQFYKLQT 60
tr|O35086|O35086          MRALGAVVTLLLWGQLFAVDLSNDAMDTADDSCPKPPEIENGYVEHLVRYRC-QHYRLRT 59
sp|P19006|HPT_CANFA       ----------------EDTGSEATENTEVSLPKPPVIENGYVEHMIRYQCKPFYKLRT 42
                                            : ..:. :  : . **** * :**** :**:*  .*:*::

sp|P00737|HPT1_HUMAN      EGDGVYTLN---------------------------------------------------- 69
sp|P00738|HPT2_HUMAN      EGDGVYTLNDKKQWINKAVGDKLPECEADDGCPKPPEIAHGYVEHSVRYQCKNYYKLRTE 120
sp|P50417|HPT_ATEGE       EGDGVYTLN---------------------------------------------------- 69
tr|Q60574|Q60574          EGDGVYTLN---------------------------------------------------- 69
tr|Q61646|Q61646          EGDGVYTLN---------------------------------------------------- 69
sp|Q62558|HPT_MUSSA       EGDGVYTLN---------------------------------------------------- 69
sp|P06866|HPT_RAT         EGDGIYTLN---------------------------------------------------- 69
tr|O35086|O35086          EGDGVYTLN---------------------------------------------------- 68
sp|P19006|HPT_CANFA       EGDGVYTLN---------------------------------------------------- 51
                          ****:****

sp|P00737|HPT1_HUMAN      --------NEKQWINKAVGDKLPECEAVCGKPKNPANPVQRILGGHLDAKGSFPWQAKMV 121
sp|P00738|HPT2_HUMAN      GDGVYTLNNEKQWINKAVGDKLPECEAVCGKPKNPANPVQRILGGHLDAKGSFPWQAKMV 180
sp|P50417|HPT_ATEGE       --------NEKQWTNKAVGDKLPECEAVCGKPKNPANPVQRILGGHLDAKGSFPWQAKMV 121
tr|Q60574|Q60574          --------DEKQWMNTVAGEKLPECEAVCGKPKHPVDQVQRIIGGSMDAKGSFPWQAKMI 121
tr|Q61646|Q61646          --------DEKQWVNTVAGEKLPECEAVCGKPKHPVDQVQRIIGGSMDAKGSFPWQAKMI 121
sp|Q62558|HPT_MUSSA       --------DEKQWVNTAAGEKLPECEAVCGKPKHPVVQVQRIIGGSMDAKGSFPWQAKMI 121
sp|P06866|HPT_RAT         --------SEKQWVNPAAGDKLPKCEAVCGKPKHPVDQVQRIIGGSMDAKGSFPWQAKMI 121
tr|O35086|O35086          --------SEKQWVNTAAGERLPECEAVCGKPKHPVDQVQRIIGGSLDAKGSFPWQAKMV 120
sp|P19006|HPT_CANFA       --------SEKHWINKAVGEKLPECEAVCGKPKNPVDQVQRIMGGSVDAKGSFPWQAKMV 103
                                  .**:* * ..*::**;**********:*.  ****;** :**********;

sp|P00737|HPT1_HUMAN      SHRNLTTGATLINEQWLLTTAKNLFLNHSENATAKDIAPTLTLYVGKKQLVEIEKVVLHP 181
sp|P00738|HPT2_HUMAN      SHRNLTTGATLINEQWLLTTAKNLFLNHSENATAKDIAPTLTLYVGKKQLVEIEKVVLHP 240
sp|P50417|HPT_ATEGE       SRRNLTTGATLINEQWLLTTAKNLFLNHSENATAKDIAPTLTLYVGKNQLVEIEKVVLYP 181
tr|Q60574|Q60574          SRHGLTTGATLISDQWLLTTAKNLFLNHSETASGKDIAPTLTLYVGKNQLVEIEKVILHP 181
tr|Q61646|Q61646          SRHGLTTGATLISDQWLLTTAKNLFLNHSETASAKDITPTLTLYVGKNQLVEIEKVVLHP 181
sp|Q62558|HPT_MUSSA       SRRGLTTGATLISDQWLLTTAKNLFLNHSETASAKDIAPTLTLYVGKNQLVEIEKVVLHP 181
sp|P06866|HPT_RAT         SRRGLTTGATLISDQWLLTTAQNLFLNHSENATAKDIAPTLTLYVGKNQLVEIEKVVLHP 181
tr|O35086|O35086          SRHELITGATLISDQWLLTTAKNLFLNHSEDATSKDIAPTLKLYVGKNQPVEIEKVVIHP 180
sp|P19006|HPT_CANFA       SHRNLTSGATLINEQWLLTTAKNLFLGHKDDAKANDIAPTLKLYVGKNQLVEVEKVVLHP 163
                          *:* * :*****.:*******:****.*.: *...**:***.***** * **:***:::*

sp|P00737|HPT1_HUMAN      NYSQVDIGLIKLKQKVSVNERVMPICLPSKDYAEVGRVGYVSGWGRNANFKFTDMLKYVM 241
sp|P00738|HPT2_HUMAN      NYSQVDIGLIKLKQKVSVNERVMPICLPSKDYAEVGRVGYVSGWGRNANFKFTDMLKYVM 300
sp|P50417|HPT_ATEGE       NYSQVDIGLIKLKDKVPVNERVMPICLPSKDYAEVGRVGYVSGWGRNANFKFTDHLKYVM 241
tr|Q60574|Q60574          NHSVVDIGLIKLKQRVLVTERVMPICLPSKDYVAPGRVGYVSGWGRNQDFRFTDRLKYVM 241
tr|Q61646|Q61646          NHSVVDIGLIKLKQRVLVTERVMPICLPSKDYIAPGRVGYVSGWGRNANFRFTDRLKYVM 241
sp|Q62558|HPT_MUSSA       NHSVVDIGLIKLKQRVLVTERVMPICLPSKDYVAPGRVGYLSGWGRNVNFRFTERFKYVM 241
sp|P06866|HPT_RAT         NRSVVDIGLIKLKQKVLVTEKVMPICLPSKDYVAPGRMGYVSGWGRNVNFRFTERLKYVM 241
tr|O35086|O35086          NRSVVDIGVIKLKQKVPVNERVMPICLPSKDYIAPGRMGYVSGWGRNANFRFTDRLKYVM 240
sp|P19006|HPT_CANFA       DYSKVDIGLIKLKQKVPIDERVMPICLPSKDYAEVGRIGYVSGWGRNSNFNFTELLKYVM 223
                          : * ****;***:;:* : *:**********   **:**:****** :*.**; ;****

sp|P00737|HPT1_HUMAN      LPVADQDQCIRHYEGSTVPEKKTPKSPVGVQPILNEHTFCAGMSKYQEDTCYGDAGSAFA 301
sp|P00738|HPT2_HUMAN      LPVADQDQCIRHYEGSTVPEKKTPKSPVGVQPILNEHTFCAGMSKYQEDTCYGDAGSAFA 360
sp|P50417|HPT_ATEGE       LPVADQYQCVKHYEGSTVPEKKTPKSPVGQQPILNEHTFCAGMSKYQEDTCYGDAGSAFA 301
tr|Q60574|Q60574          LPVADQDKCVVHYEKSTVPEKKNPTSPVGVQPILNEHTFCAGLTKYEEDTCYGDAGSAFA 301
tr|Q61646|Q61646          LPVADQDKCVVHYENSTVPEKKNLTSPVGVQPILNEHTFCAGLTKYQEDTCYGDAGSAFA 301
sp|Q62558|HPT_MUSSA       LPVADQDKCVVHYENSTVPEKKNFTSPVGVQPILNEHTFCVGLSRYQEDTCYGDAGSAFA 301
sp|P06866|HPT_RAT         LPVADQEKCELHYEKSTVPEKKGAVTPVGVQPILNEHTFCAGLTKYEEDTCYGDAGSAFA 301
tr|O35086|O35086          LPVADQDSCMLHYEGSTVPEKESGKSVGVQPILNERTFCAGMTKYQEDTCYGDAGSAFA 300
sp|P19006|HPT_CANFA       LPVADQDKCVQHYEGSTVPEKKSPKSPVGVQPILNEHTFCAGMSKFQEDTCYGDAGSAFA 283
                          ****** .*  *** ******;  ;.** *****;****.*;;;;:**************
```

Figure 4b

```
sp|P00737|HPT1_HUMAN      VHDLEEDTWYATGILSFDKSCAVAEYGVYVKVTSIQDWVQKTIAEN 347
sp|P00738|HPT2_HUMAN      VHDLEEDTWYATGILSFDKSCAVAEYGVYVKVTSIQDWVQKTIAEN 406
sp|P50417|HPT_ATEGE       VHDLEEDTWYAAGILSFDKSCGVAEYGVYVKATSIQDWVQKTIAEN 347
tr|Q60574|Q60574          IHDMEEDTWYAAGILSFDKSCAVAEYGVYVRATDLKDWVQETMAKN 347
tr|Q61646|Q61646          IHDMEEDTWYAAGILSFDKSCAVAEYGVYVRATDLKDWVQETMAKN 347
sp|Q62556|HPT_MUSSA       IHDMEEDTWYAAGILSFDKSCAVAEYGVYVRATDLKDWVQETMAKK 347
sp|P06866|HPT_RAT         VHDTEEDTWYAAGILSFDKSCAVAEYGVYVKATDLKDWVQETMAKN 347
tr|O35086|O35086          IHDLEQDTWYAAGILSFDKSCSVAEYGVYVKVNSFLDWIQETMAKN 346
sp|P19006|HPT_CANFA       VHDQDEDTWYAAGILSFDKSCTVAEYGVYVKVPSVLAWVQETIAGN 329
                          :**  ::*** *;********** ********:. ..  *:*:=:* :
```

Figure 5a

```
CD163                    MVLLEDSGSADFRRHFVNLSPFTITVVLLLSACFVTSSLGGTDKELRLVDGENKCSGRVE  60
CD163 cyt. Var 1         MVLLEDSGSADFRRHFVNLSPFTITVVLLLSACFVTSSLGGTDKELRLVDGENKCSGRVE  60
CD163 cyt. var 2         MVLLEDSGSADFRRHFVNLSPFTITVVLLLSACFVTSSLGGTDKELRLVDGENKCSGRVE  60
CD163 ext. cell. Var.    MVLLEDSGSADFRRHFVNLSPFTITVVLLLSACFVTSSLGGTDKELRLVDGENKCSGRVE  60
                         ************************************************************

tr|Q07898|Q07898         VKVQEEWGTVCNNGWSMEAVSVICNQLGCPTAIKAPGWANSSAGSGRIWMDHVSCRGNES 120
tr|Q07901|Q07901         VKVQEEWGTVCNNGWSMEAVSVICNQLGCPTAIKAPGWANSSAGSGRIWMDHVSCRGNES 120
tr|Q07900|Q07900         VKVQEEWGTVCNNGWSMEAVSVICNQLGCPTAIKAPGWANSSAGSGRIWMDHVSCRGNES 120
tr|Q07899|Q07899         VKVQEEWGTVCNNGWSMEAVSVICNQLGCPTAIKAPGWANSSAGSGRIWMDHVSCRGNES 120
                         ************************************************************

tr|Q07898|Q07898         ALWDCKHDGWGKHSNCTHQQDAGVTCSDGSNLEMRLTRGGNMCSGRIEIKFQGRWGTVCD 180
tr|Q07901|Q07901         ALWDCKHDGWGKHSNCTHQQDAGVTCSDGSNLEMRLTRGGNMCSGRIEIKFQGRWGTVCD 180
tr|Q07900|Q07900         ALWDCKHDGWGKHSNCTHQQDAGVTCSDGSNLEMRLTRGGNMCSGRIEIKFQGRWGTVCD 180
tr|Q07899|Q07899         ALWDCKHDGWGKHSNCTHQQDAGVTCSDGSNLEMRLTRGGNMCSGRIEIKFQGRWGTVCD 180
                         ************************************************************

tr|Q07898|Q07898         DNFNIDHASVICRQLECGSAVSFSGSSNFGEGSGPIWFDDLICNGNESALWNCKHQGWGK 240
tr|Q07901|Q07901         DNFNIDHASVICRQLECGSAVSFSGSSNFGEGSGPIWFDDLICNGNESALWNCKHQGWGK 240
tr|Q07900|Q07900         DNFNIDHASVICRQLECGSAVSFSGSSNFGEGSGPIWFDDLICNGNESALWNCKHQGWGK 240
tr|Q07899|Q07899         DNFNIDHASVICRQLECGSAVSFSGSSNFGEGSGPIWFDDLICNGNESALWNCKHQGWGK 240
                         ************************************************************

tr|Q07898|Q07898         MNCDHAEDAGVICSKGADLSLRLVDGVTECSGRLEVRFQGEWGTICDDGWDSYDAAVACK 300
tr|Q07901|Q07901         HNCDHAEDAGVICSKGADLSLRLVDGVTECSGRLEVRFQGEWGTICDDGWDSYDAAVACK 300
tr|Q07900|Q07900         MNCDHAEDAGVICSKGADLSLRLVDGVTECSGRLEVRFQGEWGTICDDGWDSYDAAVACK 300
tr|Q07899|Q07899         HNCDHAEDAGVICSKGADLSLRLVDGVTECSGRLEVRFQGEWGTICDDGWDSYDAAVACK 300
                         ************************************************************

tr|Q07898|Q07898         QLGCPTAVTAIGRVNASKGFGHIWLDSVSCQGHEPAVWQCKHHEWGKHYCNHNEDAGVTC 360
tr|Q07901|Q07901         QLGCPTAVTAIGRVNASKGFGHIWLDSVSCQGHEPAVWQCKHHEWGKHYCNHNEDAGVTC 360
tr|Q07900|Q07900         QLGCPTAVTAIGRVNASKGFGHIWLDSVSCQGHEPAVWQCKHHEWGKHYCNHNEDAGVTC 360
tr|Q07899|Q07899         QLGCPTAVTAIGRVNASKGFGHIWLDSVSCQGHEPAVWQCKHHEWGKHYCNHNEDAGVTC 360
                         ************************************************************

tr|Q07898|Q07898         SDGSDLELRLRGGGSRCAGTVEVEIQRLLGKVCDRGWGLKEADVVCRQLGCGSALKTSYQ 420
tr|Q07901|Q07901         SDGSDLELRLRGGGSRCAGTVEVEIQRLLGKVCDRGWGLKEADVVCRQLGCGSALKTSYQ 420
tr|Q07900|Q07900         SDGSDLELRLRGGGSRCAGTVEVEIQRLLGKVCDRGWGLKEADVVCRQLGCGSALKTSYQ 420
tr|Q07899|Q07899         SDGSDLELRLRGGGSRCAGTVEVEIQRLLGKVCDRGWGLKEADVVCRQLGCGSALKTSYQ 420
                         ************************************************************

tr|Q07898|Q07898         VYSKIQATNTWLFLSSCNGNETSLWDCKNWQWGGLTCDHYEEAKITCSAHREPRLVGGDI 480
tr|Q07901|Q07901         VYSKIQATNTWLFLSSCNGNETSLWDCKNWQWGGLTCDHYEEAKITCSAHREPRLVGGDI 480
tr|Q07900|Q07900         VYSKIQATNTWLFLSSCNGNETSLWDCKNWQWGGLTCDHYEEAKITCSAHREPRLVGGDI 480
tr|Q07899|Q07899         VYSKIQATNTWLFLSSCNGNETSLWDCKNWQWGGLTCDHYEEAKITCSAHREPRLVGGDI 480
                         ************************************************************

tr|Q07898|Q07898         PCSGRVEVKHGDTWGSICDSDFSLEAASVLCRELQCGTVVSILGGAHFGEGNGQIWAEEF 540
tr|Q07901|Q07901         PCSGRVEVKHGDTWGSICDSDFSLEAASVLCRELQCGTVVSILGGAHFGEGNGQIWAEEF 540
tr|Q07900|Q07900         PCSGRVEVKHGDTWGSICDSDFSLEAASVLCRELQCGTVVSILGGAHFGEGNGQIWAEEF 540
tr|Q07899|Q07899         PCSGRVEVKHGDTWGSICDSDFSLEAASVLCRELQCGTVVSILGGAHFGEGNGQIWAEEF 540
                         ************************************************************

tr|Q07898|Q07898         QCEGHESHLSLCPVAPRPEGTCSHSRDVGVVCS---------------------------- 573
tr|Q07901|Q07901         QCEGHESHLSLCPVAPRPEGTCSHSRDVGVVCSSKTQXTSLIGSYTVKGTGLGSHSCLFL 600
tr|Q07900|Q07900         QCEGHESHLSLCPVAPRPEGTCSHSRDVGVVCS---------------------------- 573
tr|Q07899|Q07899         QCEGHESHLSLCPVAPRPEGTCSHSRDVGVVCS---------------------------- 573
                         ***************************

tr|Q07898|Q07898         ------RYTEIRLVNGKTPCEGRVELKTLGAWGSLCNSHWDIEDAHVLCQQLKCGVALST 627
tr|Q07901|Q07901         KPCLLPGYTEIRLVNGKTPCEGRVELKTLGAWGSLCNSHWDIEDAHVLCQQLKCGVALST 660
tr|Q07900|Q07900         ------RYTEIRLVNGETPCEGRVELKTLGAWGSLCNSHWDIEDAHVLCQQLKCGVALST 627
tr|Q07899|Q07899         ------RYTEIRLVNGKTPCEGRVELKTLGAWGSLCNSHWDIEDAHVLCQQLKCGVALST 627
                                ************************************************
```

Figure 5b

```
tr|Q07898|Q07898    PGGARFGKGNGQIWRHMFHCTGTEQHMGDCPVTALGASLCPSEQVASVICSGNQSQTLSS 687
tr|Q07901|Q07901    PGGARFGKGNGQIWRHMFHCTGTEQHMGDCPVTALGASLCPSEQVASVICSGNQSQTLSS 720
tr|Q07900|Q07900    PGGARFGKGNGQIWRHMFHCTGTEQHMGDCPVTALGASLCPSEQVASVICSGNQSQTLSS 687
tr|Q07899|Q07899    PGGARFGKGNGQIWRHMFHCTGTEQHMGDCPVTALGASLCPSEQVASVICSGNQSQTLSS 687
                    ************************************************************

tr|Q07898|Q07898    CNSSSLGPTRPTIPEESAVACIESGQLRLVNGGGRCAGRVEIYHEGSWGTICDDSWDLSD 747
tr|Q07901|Q07901    CNSSSLGPTRPTIPEESAVACIESGQLRLVNGGGRCAGRVEIYHEGSWGTICDDSWDLSD 780
tr|Q07900|Q07900    CNSSSLGPTRPTIPEESAVACIESGQLRLVNGGGRCAGRVEIYHEGSWGTICDDSWDLSD 747
tr|Q07899|Q07899    CNSSSLGPTRPTIPEESAVACIESGQLRLVNGGGRCAGRVEIYHEGSWGTICDDSWDLSD 747
                    ************************************************************

tr|Q07898|Q07898    AHVVCRQLGCGEAINATGSAHFGEGTGPIWLDEMKCNGKESRIWQCHSHGWGQQNCRHKE 807
tr|Q07901|Q07901    AHVVCRQLGCGEAINATGSAHFGEGTGPIWLDEMKCNGKESRIWQCHSHGWGQQNCRHKE 840
tr|Q07900|Q07900    AHVVCRQLGCGEAINATGSAHFGEGTGPIWLDEMKCNGKESRIWQCHSHGWGQQNCRHKE 807
tr|Q07899|Q07899    AHVVCRQLGCGEAINATGSAHFGEGTGPIWLDEMKCNGKESRIWQCHSHGWGQQNCRHKE 807
                    ************************************************************

tr|Q07898|Q07898    DAGVICSEFMSLRLTSEASREACAGRLEVFYNGAWGTVGKSSMSETTVGVVCRQLGCADK 867
tr|Q07901|Q07901    DAGVICSEFMSLRLTSEASREACAGRLEVFYNGAWGTVGKSSMSETTVGVVCRQLGCADK 900
tr|Q07900|Q07900    DAGVICSEFMSLRLTSEASREACAGRLEVFYNGAWGTVGKSSMSETTVGVVCRQLGCADK 867
tr|Q07899|Q07899    DAGVICSEFMSLRLTSEASREACAGRLEVFYNGAWGTVGKSSMSETTVGVVCRQLGCADK 867
                    ************************************************************

tr|Q07898|Q07898    GKINPASLDKAMSIPMWVDNVQCPKGPDTLWQCPSSPWEKRLASPSEETWITCDNKIRLQ 927
tr|Q07901|Q07901    GKINPASLDKAMSIPMWVDNVQCPKGPDTLWQCPSSPWEKRLASPSEETWITCDNKIRLQ 960
tr|Q07900|Q07900    GKINPASLDKAMSIPMWVDNVQCPKGPDTLWQCPSSPWEKRLASPSEETWITCDNKIRLQ 927
tr|Q07899|Q07899    GKINPASLDKAMSIPMWVDNVQCPKGPDTLWQCPSSPWEKRLASPSEETWITCDNKIRLQ 927
                    ************************************************************

tr|Q07898|Q07898    EGPTSCSGRVEIWHGGSWGTVCDDSWDLDDAQVVCQQLGCGPALKAFKEAEFGQGTGPIW 987
tr|Q07901|Q07901    EGPTSCSGRVEIWHGGSWGTVCDDSWDLDDAQVVCQQLGCGPALKAFKEAEFGQGTGPIW 1020
tr|Q07900|Q07900    EGPTSCSGRVEIWHGGSWGTVCDDSWDLDDAQVVCQQLGCGPALKAFKEAEFGQGTGPIW 987
tr|Q07899|Q07899    EGPTSCSGRVEIWHGGSWGTVCDDSWDLDDAQVVCQQLGCGPALKAFKEAEFGQGTGPIW 987
                    ************************************************************

tr|Q07898|Q07898    LNEVKCKGNESSLWDCPARRWGHSECGHKEDAAVNCTDISVQKTPQKATTGRSSRQSSFI 1047
tr|Q07901|Q07901    LNEVKCKGNESSLWDCPARRWGHSECGHKEDAAVNCTDISVQKTPQKATTGRSSRQSSFI 1080
tr|Q07900|Q07900    LNEVKCKGNESSLWDCPARRWGHSECGHKEDAAVNCTDISVQKTPQKATTGRSSRQSSFI 1047
tr|Q07899|Q07899    LNEVKCKGNESSLWDCPARRWGHSECGHKEDAAVNCTDISVQKTPQKATTGRSSRQSSFI 1047
                    ************************************************************

tr|Q07898|Q07898    AVGILGVVLLAIFVALFFLTKKRRQRQRLAVSSRGENLVHQIQYREMNSCLNADDLDLMN 1107
tr|Q07901|Q07901    AVGILGVVLLAIFVALFFLTKKRRQRQRLAVSSRGENLVHQIQYREMNSCLNADDLDLMN 1140
tr|Q07900|Q07900    AVGILGVVLLAIFVALFFLTKKRRQRQRLAVSSRGENLVHQIQYREMNSCLNADDLDLMN 1107
tr|Q07899|Q07899    AVGILGVVLLAIFVALFFLTKKRRQRQRLAVSSRGENLVHQIQYREMNSCLNADDLDLMN 1107
                    ************************************************************

tr|Q07898|Q07898    SSG----GHSEPH---------------------------------- 1116
tr|Q07901|Q07901    SSG----GHSEPH---------------------------------- 1149
tr|Q07900|Q07900    SSGLWVLGGSIAQGFRSVAAVEAQTFYFDKQLKKSKNVIGSLDAYNGQE 1156
tr|Q07899|Q07899    SSE-----NSHESADFSAAELISVSKFLPISGMSKEAILSHTEKENGNL 1151
                    **       *
```

Figure 6a

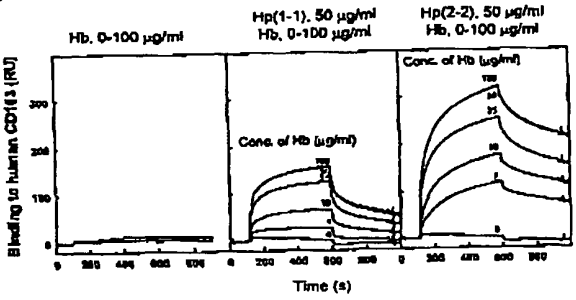

Figure 6b

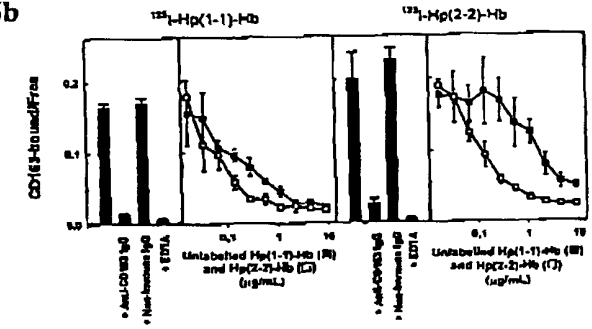

Figure 7a

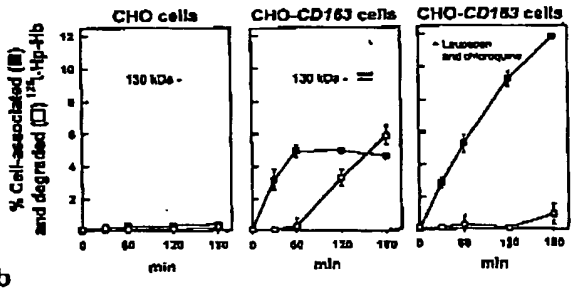

Figure 7b